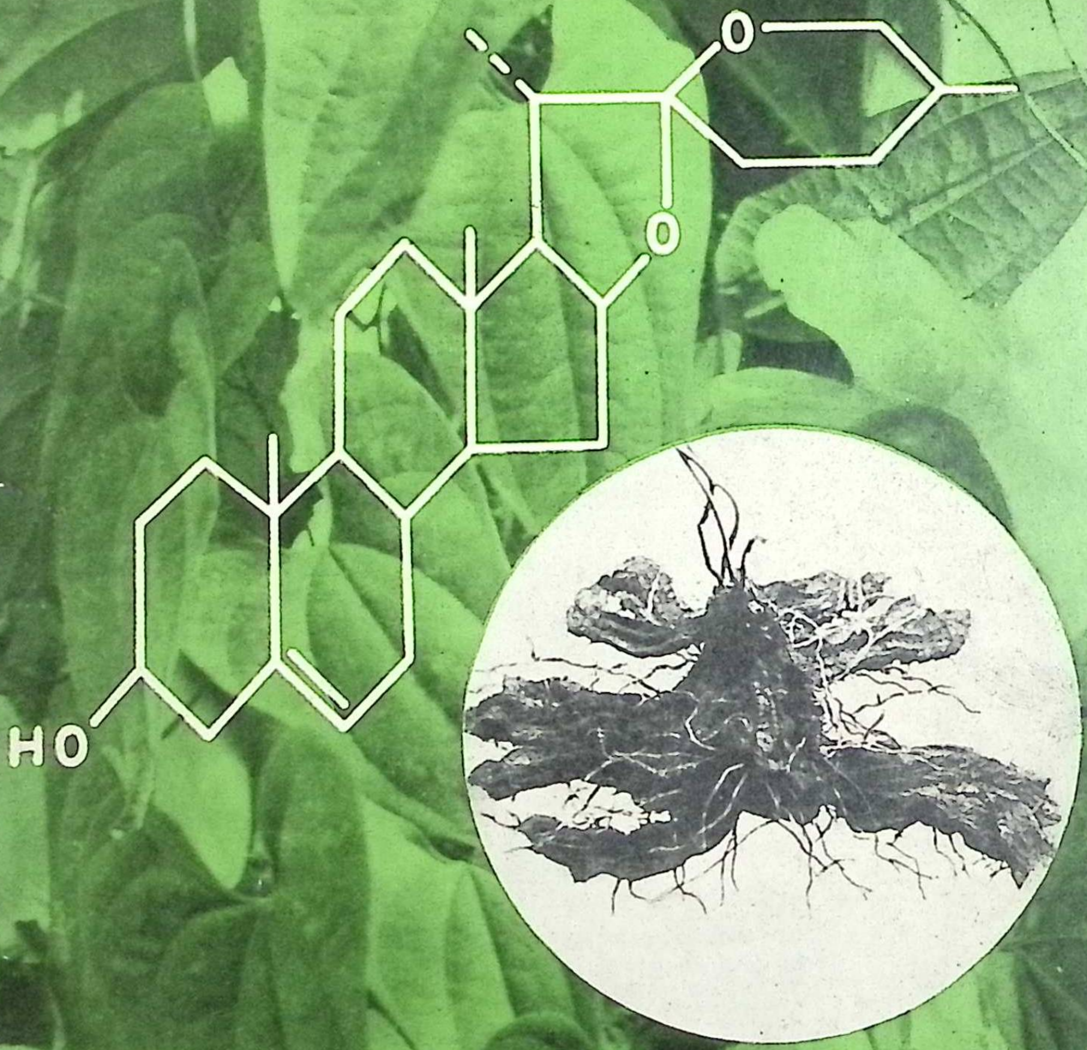
DIOSGENIN AND OTHER STEROID DRUG PRECURSORS



PUBLICATIONS & INFORMATION DIRECTORATE, CSIR,
Hillside Road, New Delhi-110012

2346

DIOSGENIN
AND
OTHER STEROID DRUG
PRECURSORS

ADVISORY COMMITTEE

C.K. Atal
Director
Regional Research Laboratory
Jammu-Tawi

Y.K. Hamied
Managing Director
CIPLA
Bombay

T.N. Khoshoo
Director
National Botanical Research Institute
Lucknow

Nitya Anand
Director
Central Drug Research Institute
Lucknow

Y.R. Chadha
Chief Editor
Publications & Information Directorate
New Delhi

J6
N79

000143

DIOSGENIN
AND
OTHER STEROID DRUG PRECURSORS

L.V.Asolkar (Miss)
Y.R. Chadha

assisted by
P.S. Rawat

PUBLICATIONS & INFORMATION DIRECTORATE, CSIR
New Delhi-110012
1979

© 1979

PUBLICATIONS & INFORMATION DIRECTORATE (CSIR)
HILLSIDE ROAD, NEW DELHI - INDIA

This book shall not be sold outside India by anyone,
without the prior permission of the publisher

Price: Rs 32.00 $ 12.00 £ 5.50

Published and printed by the Publications & Information Directorate, New Delhi, at its Photocomposition-cum-Printing Unit

Reprinted 1982

PREFACE

Diosgenin has been the foremost starting material in the manufacture of steroid drugs for nearly three decades. The situation changed in the mid 70's when the other drug precursors came into prominence. The world steroid drug industry, till then, was based mainly on Mexican diosgenin extracted from tubers of wild *Dioscorea* spp. The ever-growing demand for steroid drugs and hence for diosgenin, coupled with dwindling wild sources of this precursor and continued spurt in its prices, led to frenzied research efforts all over the world to develop new sources of diosgenin, and also of alternate precursors, with better routes of production and cheaper end-products. As a result, diosgenin is today facing stiff competition from other precursors like stigmasterol, cholesterol, sitosterol, and hecogenin. The development of these steroid precursors is one of the finest success stories of modern science and technology.

An attempt is made in this monograph to present a comprehensive and up-to-date, albeit concise, account of all these and other developments in the steroid industry. It is, however, difficult to keep track of all the advances that are taking place in this rapidly developing field. Based primarily on published literature, the monograph also draws on extensive personal communications and discussions with experts in the field. It is intended to serve as a compendium on the various facets of the steroid industry, with special reference to India. Diosgenin is dealt with here in greater details than the other steroid precursors because it is still the mainstay of the Indian steroid industry.

The work of compilation of information was undertaken by Miss L.V. Asolkar of this Directorate, who accomplished it with commendable thoroughness. It was truly a tremendous task which involved voluminous literature survey, extensive correspondence with experts, and visits to some of the steroid drug manufacturing firms. She was ably assisted by P.S. Rawat and other colleagues in the Wealth of India Section in the collection of information, and checking and standardization of citations. K. Kashyapa, a senior colleague, was good enough to go through the botanical aspects of the monograph.

The neat line-drawings are by our artist, P.R. Mehta, who has taken great pains in drawing the complex molecular flow-sheets. The pleasing cover design is by our senior artist, S.K. Das Gupta; the old-fashioned ring structure of diosgenin may perhaps appear somewhat irritating to a chemist. Unstinted assistance has also been rendered by the Production and Reference Sections. The monograph was printed at our newly set-up Photocomposition-cum-Printing Unit. The Chief Editor thanks them all for their enthusiastic co-operation.

The Chief Editor places on record his deep gratitude to the members of the Advisory Committee for their constant guidance and valuable suggestions, and, for scrutiny of the various Chapters of this monograph: Dr. Nitya Anand, CDRI, Lucknow (Chapters 1, 3 & 4), Dr. C.K. Atal, RRL, Jammu (Chapter 2); and Dr. Y.K. Hamied, CIPLA, Bombay (Chapters 4, 5 & 6). He is especially grateful to Dr. Y.K. Hamied for providing latest information on various aspects of steroid precursors, and to Dr. T.N. Khoshoo for clarifying certain doubts regarding dioscoreas.

A number of senior scientists and institutions in India and abroad have assisted in making available valuable information and photographs, for which the Chief Editor is grateful to them. In particular, he thanks the following experts: Dr. H.C. Chaṭurvedi (NBRI, Lucknow) for the write-up on "Tissue Culture" in Chapter 2, Dr. R.K. Bammi (CIPLA, Bangalore), Dr. M.C. Tyagi (IARI, New Delhi), Dr. V.R. Gadwal (Glaxo, Bombay), and Dr. Y.K. Sarin (RRL, Jammu). Of the foreign experts, the Chief Editor is extremely thankful to Dr. Norman Applezweig for his esteemed cooperation. His prestigious 'Steroid Drugs' and his lecture on 'Dioscorea—the pill crop' proved invaluable in writing this monograph. Thanks are also due to the Director, Tropical Products Institute, London for supplying useful documents.

Publications & Information Directorate, CSIR
Hillside Road, New Delhi - 110012

Y.R. Chadha
Chief Editor

CONTENTS

PREFACE ... v

CHAPTER 1 INTRODUCTION ... 1-10

- Chemical Background ... 1
- History of Steroid Development ... 4
  - Discovery of Diosgenin ... 5
  - Discovery of Cortisone ... 5
- Trends ... 7
  - Other precursors ... 7
- Position in India ... 8
- References ... 9

CHAPTER 2 SOURCES OF DIOSGENIN ... 11-59

- Dioscoreas ... 11
- Wild Dioscoreas ... 12
  - D. deltoidea ... 13
  - D. Prazeri ... 18
- Dioscoreas under Cultivation ... 21
  - Agronomy ... 22
  - D. floribunda ... 26
  - D. composita ... 31
  - D. deltoidea ... 34
  - D. prazeri ... 36
  - Economics of Cultivation ... 37
- Genetics, Breeding and Plant Improvement ... 39
- Tissue Culture ... 44
- Other Genera ... 47
  - Costus speciosus ... 47
  - Kallstroemia pubescens ... 51
  - Trigonella foenum-graecum ... 51
  - Balanites aegyptiaca ... 53
- References ... 56

CHAPTER 3 EXTRACTION AND ASSAY PROCEDURES OF DIOSGENIN ... 60-92

- Extraction Methods ... 61
  - Alcoholic Extraction Method ... 61
  - Acid Hydrolysis Method ... 63
  - Fermentation-cum-Acid Hydrolysis Method 64
  - Other Methods ... 65
  - Machinery and Chemicals Required ... 67
- Economics of Diosgenin Extraction in India ... 68
  - Cultivated Tubers ... 68
  - Wild Tubers ... 71
- Isolation and Identification ... 71

Assay Procedures ... 75
  Gravimetric Methods ... 76
  Colorimetric Methods ... 77
  GLC Methods ... 79
  Densitometric TLC Methods ... 81
  IR Spectrophotometry ... 82
  P–TLC—IR Spectrophotometry ... 83
  Column Chromatography—IR Spectrophotometry ... 86
References ... 90
CHAPTER 4 DIOSGENIN CONVERSION TO STEROID DRUGS AND USES ... 93-111
Conversions ... 93
  16-DPA ... 93
  Steroid Classication ... 95
  Microbiological Conversions ... 96
  Corticosteroids ... 97
  Pregnenes ... 98
  Androstenes ... 99
  Estrenes and 19 Nor Steroids ... 99
Uses of Steroid Drugs ... 106
References ... 110
CHAPTER 5 STEROID DRUG PRECURSORS OTHER THAN DIOSGENIN ... 112-45
Solasodine ... 112
  Solanum khasianum ... 113
  Economics of S. khasianum Cultivation ... 122
  Other Solanum Species ... 123
  Prospects for Solasodine ... 124
Hecogenin ... 124
  Extraction of Hecogenin ... 128
  Prospects for Hecogenin ... 131
Stigmasterol ... 132
Sitosterols ... 135
Cholesterol ... 137
Deoxycholic and Cholic Acids ... 139
Ergosterol ... 142
Vegetable Pregnane Steroids ... 143
Miscellaneous Precursors ... 144
References ... 145
CHAPTER 6 PRODUCTION, REQUIREMENTS AND TRADE 146-56
Diosgenin ... 146
Other Steroid Precursors ... 148
Requirements of Steroid Precursors ... 149
  Indian Requirements ... 149

CONTENTS

Trade ... 150
  Prices of Diosgenin ... 150
  Imports and Exports ... 152
Trends ... 152
  Steroid Starting Materials ... 152
  Trends in India ... 153
  Synthetic Steroids ... 154
References ... 156

APPENDIX I Plant Species Reported to Contain diosgenin 157-59

APPENDIX II Glossary of Botanical and Agricultural Terms 160-62

APPENDIX III Diosgenin Extraction Centres in India 163

INDEX ... 165-70

LIST OF ILLUSTRATIONS

PLATES

Plate		Facing page
I	Pieces of dried rhizomes of wild Dioscorea deltiodea	17
II	Dioscorea tubers from cultivated plants	31
III	Steroid conversion plants	98
IV	Multipurpose steroid plant	99

TEXT FIGURES

Fig.		Page
1.1	Steroid nucleus and some important steroid drug precursors ...	2
1.2	Some naturally occurring sex hormones ...	3
1.3	Structure of cortisone ...	5
2.1	*Dioscorea deltoidea*: Plant parts ...	14
2.2	Dried rhizome of wild *Dioscorea deltoidea* ...	15
2.3	*Dioscorea prazeri*: Plant parts ...	19
2.4	Rhizome of *Dioscorea prazeri* ...	20
2.5	Dioscorea floribunda: Portions of a tuber ...	27
2.6	Dioscorea floribunda: Cultivated plant ...	28
2.7	*Dioscorea composita*: Plant parts ...	32
2.8	*Dioscorea composita* under experimental cultivation ...	33
2.9	Cultivated *Dioscorea deltoidea* with tubers ...	35
2.10	Tubers of *Dioscorea deltoidea* after 2 years' growth ...	36
2.11	Different shapes of the tubers of wild *Dioscorea* spp., diagrammatic ...	39
2.12	Clonal propagation of *Dioscorea floribunda* through *in vitro* culture ...	45
2.13	Tissue culture of *Dioscorea deltoidea* ...	46
2.14	*Costus speciosus*: the whole plant ...	48
2.15	*Costus speciosus*: Tubers harvested from 18 month-old plant ...	49
2.16	*Balanites aegyptiaca*: Fruiting branch and dried fruits ...	54
2.17	*Balanites aegyptiaca*: Fruit and its transverse section ...	55
3.1	Dioscin, a diosgenin glycoside ...	62
3.2	Extraction methods for diosgenin from different sources ...	66
3.3	Diosgenin extraction unit, CIPLA, Vikhroli, Bombay ...	68
3.4	Structure of diosgenin ...	72
3.5	Infra-red spectra of diosgenin, yamogenin and their mixtures ...	74
3.6	Standard diosgenin curve for colorimetric determination ...	78
3.7	Gas chromatogram of diosgenin from *Dioscorea deltoidea* yam extract ...	79
3.8	Calibration graph of diosgenin ...	80

LIST OF ILLUSTRATIONS

Fig. Page
3.9 Gas chromatogram of *Dioscorea floribunda* yam extract ... 80
3.10 D:Y Ratio graph ... 85
4.1 Conversion of diosgenin to 16-DPA ... 93
4.2 Product pattern with intermediates and yields from diosgenin ... 94
4.3 Some stereoparents ... 96
4.4 Molecular flow-sheet of some steroid processes using diosgenin ... 100-03
4.5 Some microbiological transformations of Compound S ... 104
4.6 Some microbiological transformations of progesterone ... 105
5.1 *Solanum khasianum* var. *chatterjeeanum* ... 116
5.2 Leaf of *Solanum khasianum* var. *chatterjeeanum* ... 117
5.3 Leaf of reduced-spine mutant of *Solanum khasianum* var. *chatterjeeanum* ... 117
5.4 Fruits of *Solanum khasianum* var. *chatterjeeanum* ... 118
5.5 Molecular flow-sheet of some steroid processes using hecogenin and sterols ... 130
5.6 Product pattern with intermediates and yields from stigmasterol ... 133
5.7 Summary flow-sheet of industrial processes using steroid drug precursors other than diosgenin ... 140
5.8 Structure of ergosterol ... 142

ABBREVIATIONS FOR ORGANISATIONS

AICIP	...	All India Co-ordinated Improvement Project, New Delhi
CAZRI	...	Central Arid Zone Research Institute, Jodhpur
CDRI	...	Central Drug Research Institute, Lucknow
CIMAP	...	Central Institute for Medicinal & Aromatic Plants, Lucknow, earlier CIMPO
CIMPO	...	Central Indian Medicinal Plants Organisation, now CIMAP, Lucknow
DRL	...	Drug Research Laboratory, Jammu-Tawi
IARI	...	Indian Agricultural Research Institute, New Delhi
ICAR	...	Indian Council of Agricultural Research, New Delhi
ICMR	...	Indian Council of Medical Research, New Delhi
IIHR	...	Indian Institute of Horticultural Research, Bangalore
NBG	...	National Botanic Gardens, now NBRI, Lucknow
NBRI	...	National Botanical Research Institute, Lucknow, earlier NBG
NCL	...	National Chemical Laboratory, Pune
RRL	...	Regional Research Laboratory, Bhubaneswar; Jammu - Tawi; and Jorhat

CHAPTER 1

INTRODUCTION

Compounds which can be converted into steroid drugs are known as steroid drug precursors. Of these, diosgenin is the most important and versatile precursor and accounts for about 50 per cent of the total steroid drugs output in the world. Cholesterol, stigmasterol, sitosterols, bile acids, solasodine and hecogenin are the other steroid precursors in use. The steroid drug industry in India relies almost entirely on diosgenin as the base material.

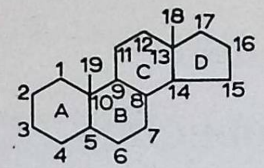
Steroid drugs can be classified into three main groups on the basis of their applications, namely corticosteroids, sex hormones and anti-fertility compounds. Corticosteroids which include cortisone, hydrocortisone, and their improved derivatives are known to possess anti-inflammatory properties and provide relief from arthritis, rheumatism, and asthma. Sex hormones, male and female, are used as substitution therapy for deficiency in natural hormones. Testosterone, one of the most potent of male sex hormones, and its modifications are used to treat testicular insufficiency and as anabolics to rebuild bodies wasted by severe illness or stunted by abnormal growth. Estradiol, estrone and progesterone, the female sex hormones, are useful in the treatment of gynaecological disorders. Along with their modifications, the female sex hormones serve as anti-fertility agents—the active ingredients of oral contraceptive pills.

These and many more steroid drugs with very many applications are produced from diosgenin and other steroid precursors.

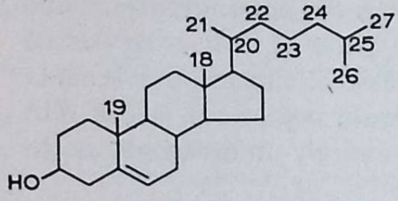
Chemical Background

Steroids are a class of organic compounds having in common a perhydrocyclopentanophenanthrene nucleus (Fig. 1.1). They include, among others, sterols, certain sapogenins, bile acids, sex hormones and adrenal cortex hormones. Some alkaloids like solasodine also belong to this class of compounds. All these compounds have the same basic structural skeleton or nucleus of four fused rings of 17 carbon atoms but they differ in chemical groups or side-chain attached to this basic skeleton and a double bond here and there in some position in the nucleus (Figs. 1.1, 1.2 & 1.3).

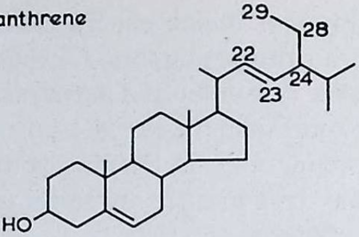
Sterols are found abundantly in nature, usually in the nonsaponifiable fraction of fats and oils of animal and plant origin. They all have a hydroxyl group at 3 position, and a double bond between 5 and 6 positions but they differ in the side-chain at 17. The sterols being used or


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
A
B
C
D

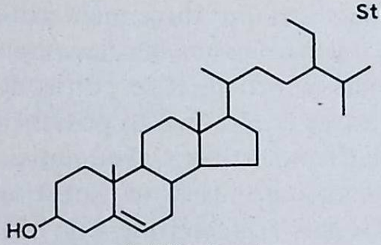
Perhydrocyclopentanophenanthrene


21
22
24
27
20
23
25
18
26
19
HO

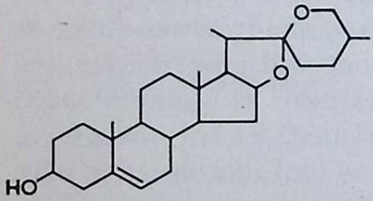
Cholesterol


29
28
22
24
23
HO

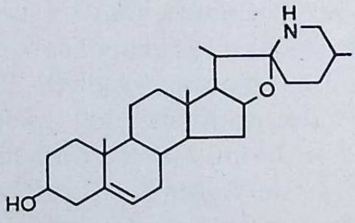
Stigmasterol



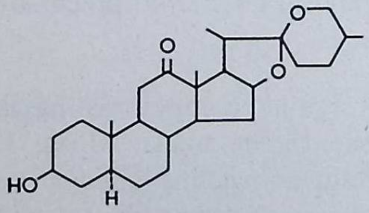
β-Sitosterol



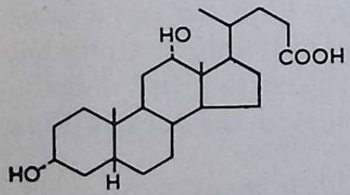
Diosgenin



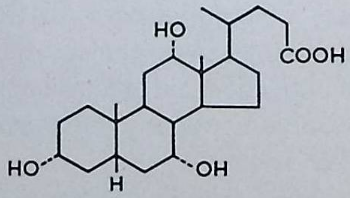
Solasodine



Hecogenin



Deoxycholic Acid


HO
COOH
HO
OH
H

Cholic Acid

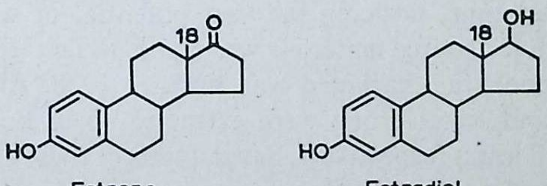
Fig. 1.1—Steroid nucleus and some important steroid drug precursors

potentially useful as steroid drug precursors are cholesterol obtained from animal fats, sitosterol and stigmasterol from vegetable oils, and ergosterol from yeast and other microbiological sources (Fig. 1.1).

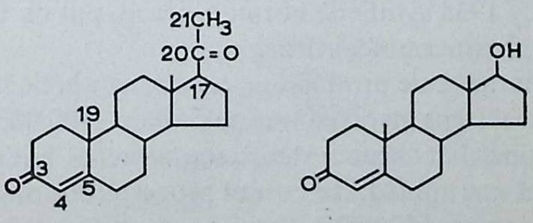
Steroidal sapogenins occur in plants. They have a spiroketal side-chain attached at positions 16 and 17; they may or may not have a double bond at 5-6. They all have a hydroxyl group at 3 and may have additional hydroxyl groups elsewhere in the nucleus. The hydroxyl groups are mostly found combined with sugars, making the compounds water-soluble and highly saponaceous. Diosgenin and hecogenin are the important sapogenins; also resembling their structure is an alkaloid solasodine (Fig. 1.1). Diosgenin is obtained entirely from the natural sources (yams of *Dioscorea* spp.) since synthetic product is not an attractive proposition commercially. Although total synthesis of a few steroid drugs has been achieved, until recently none could compete economically with partial synthesis from naturally occurring compounds, particularly diosgenin.

Bile acids are present in the bile of all animals. They have a hydroxyl group at 3 and may have additional ones attached elsewhere on the cyclopentanophenanthrene nucleus, and all have a characteristic side-chain at 17 ending in a carboxylic group. When converted into alkaline salts, these compounds show detergent-like properties resembling those of the sapogenins. Of the bile acids, cholic and deoxycholic acids (Fig. 1.1) are used for the production of steroid drugs to a limited extent.

Sex hormones (Fig. 1.2) and adrenal cortex hormones are secreted into the blood by the endocrine or ductless glands of the body exercising specific stimulatory physiological actions on other organs to which they



Estrone Estradiol



Progesterone Testosterone

Fig. 1.2—Some naturally occurring sex hormones

are carried by blood. Hormones are generally released in exceedingly small amounts. Release or non-release of the minute quantities of hormones can promote or disturb the harmonious functioning of the body. A minute change in the steroid hormone molecule may mean widely differing physiological actions. What is more, a trivial change such as presence of a methyl group or a double bond may mean difference between maleness and femaleness. The steroid hormones are synthesized from other allied compounds.

HISTORY OF STEROID DEVELOPMENT

Steroid history dates back to 1773, with the isolation of cholesterol from gall stones. There was a long dormant period before the interest in cholesterol was revived in 1903 by the study of testicular, ovarian, bile, and adrenal extracts, and the urine of a pregnant mare. At the same time, chemists were determining structure of cholesterol, bile acids and hormones. During 1929 to 1935 most of the initial difficulties were overcome and it was in this period that the isolation, characterization and structure determination of estrone, progesterone and testosterone were accomplished; structures of cholesterol and bile acids were also determined. This initial break-through in the field of steroids was achieved chiefly by the pioneering work of such names in organic chemistry as Windaus, Wieland, Butenandt and Ruzicka. Also associated with this work were pharmaceutical companies of world fame namely, Parke, Davis & Co. (USA), Scherings (Germany), Roussel Lab. (France), Organon (Holland) and CIBA (Switzerland).

Around that time, noticing the vast potential of sex hormones a vigorous search for source materials was made. In fact a crude hormone product (Estrone) was marketed way back in 1928. At first, estrone, progesterone and testosterone were extracted from horse urine, sow ovaries and bull testes respectively. Several tons of these animal products were required and it was by no means easy to obtain them. As the demand grew for hormones, the animal precursors like cholesterol and bile acids that were comparatively cheap and abundant sources at that time, came into use, and by 1938 synthetic hormones were put on the market by as many as four pharmaceutical firms.

In spite of large scale production, cost of synthetic hormones due to multiple chemical steps involved was more than $100,000 per kg. This led to the use of somewhat cheaper sterol, stigmasterol, but its availability in crystalline form was limited; the cost of progesterone obtained even from plant sterol was around $80,000. The expanding demand, prohibitive cost and limited supply of starting materials for sex hormones prompted further search.

Discovery of Diosgenin

This search proved fruitful in 1939, when Russel Marker of Pennsylvania State University discovered that the tubers of a wild Mexican plant, locally known as *cabeza de negra* (*Dioscorea mexicana*), contained a steroidal sapogenin in large amount. The sapogenin proved to be diosgenin, earlier isolated in 1936 by Ueno & Tsukamoto in a native Japanese species, *Dioscorea tokoro*. In 1940, Marker published a series of papers dealing with the breakdown of diosgenin to a number of sex hormones; Marker's method of degradation still remains the only method commercially feasible.

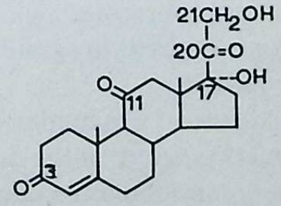
Marker could foresee the large scale exploitation of the wild Mexican yams, *cabeza de negra*, for the production of hormones. He left his academic post to set up hormone industry based on diosgenin, when Parke, Davis rejected his counsel to do so.

In 1944, Marker set up a firm Syntex, A.A. in Mexico and started producing progesterone from diosgenin bringing the price of this hormone crashing down to less than $2,000 per kg.

Discovery of Cortisone

A number of compounds including cortisone were isolated from the extracts of adrenal cortex in the period 1936-42 by as many as four groups of workers, each naming the compounds or substances differently resulting in confusion. But it was not until 1948, that cortisone was fully identified. During the World War II, the U.S. Government received information that the Germans were feeding adrenal gland extracts to their pilots. The U.S. Government approached Merck to investigate the extract. Information though proved incorrect was instrumental in the discovery, isolation and structure determination of cortisone. Clinical trials surprisingly proved its beneficial effects on patients suffering from rheumatoid arthritis. Merck set about making cortisone from bile acids and was successful in putting cortisone on the market in 1948. It was then regarded as "Wonder drug" and its demand expanded all over the world.

The structure of cortisone (Fig. 1.3) differs from progesterone in that cortisone has additional oxygen functions in 11, 17 and 21 of the progesterone molecule. Introduction of hydroxyl groups at position 17



Cortisone

Fig. 1.3—Structure of cortisone

and 21 by chemical means was not much of a problem, but introduction at 11 was a formidable task. At that time the only way in which oxygenation at 11 could be achieved was by starting synthesis from bile acids which included compounds having a hydroxyl group at position 12, which by elaborate processing could be converted into 11 keto group. Diosgenin does not contain any keto or hydroxyl group in position 11 or 12 and could not, therefore, enter the synthesis of cortisone. Thus its use was limited to the conversion of sex hormones alone.

It was obvious that bile acids could not meet the world demand and search was therefore on for a plant sapogenin having oxygen atom in position 11 or 12. This led to hecogenin which has oxygen in 12 position (see Fig. 1.1).

An outstanding advance in the steroid field came in 1949, when it was discovered by Peterson of Upjohn Co. that the enzymes could selectively hydroxylate No. 11 carbon atom of steroid molecule with astonishing ease, which hitherto involved tremendous efforts on the part of the chemists. The microbiological process at once made diosgenin a source material of choice, since progesterone obtained from it could be transformed into 11-hydroxyprogesterone.

Marker and Applezweig had in the meantime discovered that another Mexican yam, *D. composita*, locally known as *barbasco*, contained 3 to 10 times more diosgenin than *D. mexicana*. The discovery of *barbasco* as a rich source of diosgenin proved timely because the world requirement of progesterone for the synthesis of cortisone was no longer in kilos but in tonnes. At that time, progesterone commanded a price of around $1500 per kg; the new source of diosgenin and large scale operation enabled Syntex to provide Upjohn with progesterone for only $480 per kg. Upjohn later developed an efficient route to progesterone from stigmasterol, which forced the price of Mexican (Syntex) progesterone down to less than $200 per kg. It was thus possible to make available cortisone in large quantities and within the reach of the common man.

Cortisone was not free from side-effects in patients and this led to the development of improved corticoids such as hydrocortisone, prednisone and prednisolone.

Side by side with the progress in corticosteroids, a number of discoveries were made in sex hormone field as well. More potent hormones such as ethisterone, hydroxyprogesterone esters and estradiol derivatives came into the market.

Another significant step forward was made in 1952, when it was found that elimination of the 19-methyl group in the steroid molecule enhanced the hormonal activity of the compound tremendously. This resulted in the production of 19-Nor 'super-hormones' and in the introduction of highly active female sex hormones as oral contraceptives in 1955.

TRENDS

Whether it was sex hormones or corticosteroids, whether it was 19-Nors or oral contraceptives, whether it was anabolics or diuretics, diosgenin was always available and available in plenty from wild-growing dioscoreas in Mexico at almost throw-away prices. Situation changed over the years, growing world demand could not be met with wild tubers, and in spite of other diosgenin-yielding species like *D. floribunda* having been discovered, cultivation of dioscoreas was not taken seriously and prices of tubers and consequently of diosgenin went on increasing Diosgenin which ruled supreme in 50's and 60's received a big jolt in mid 70's due to continued spurt in prices.

In January 1975, Mexican Government took over the collection of *Dioscorea* tubers and set new prices and control of allocation to industry. The diosgenin manufacturers were thus faced not only with four-fold increase in the cost of raw material but also an uncertainty about its continued availability. As a result, it became increasingly difficult for steroid drugs based on diosgenin to compete with those obtained from other precursors. The diosgenin manufacturers curtailed their production owing to dwindling offtake and steroid shortage became imminent. This caused intense inflation as also an incentive to fill the vacuum by other steroid drug precursors as well as by total synthesis.

Other Precursors

The other steroid drug precursors, namely cholesterol, stigmasterol, bile acids and hecogenin, in fact the first three being predecessors of diosgenin, have never been actually the dead issues. They were always there but their individual role in steroid drug production had been reduced to insignificance by diosgenin. These steroid drug precursors with added number and considerable vigour are now coming to the fore following the decline in the production of diosgenin in Mexico.

Manufacturers using other steroid drug precursors chose to augment their sources and to improve the manufacturing processes. Thus, cholesterol, stigmasterol and hecogenin are available in almost unlimited amount, while bile acids though still in use, remain as unfavoured as ever. Sitosterols and solasodine are the new entrants. Cholesterol from wool industry and stigmasterol and sitosterols from soap industry have come into prominence not only due to their being cheap by-products but also due to the development of efficient microbiological conversions, which eliminate a number of chemical reaction steps with cheaper and better yields of intermediates and consequently of steroid drugs.

Diosgenin is now used mainly for the production of corticosteroids, where again stigmasterol, hecogenin and solasodine, especially the first two, are making their presence increasingly felt.

The Mexican Government realising its folly reduced the prices of *Dioscorea* tubers, but not before the other steroid drug precursors armed with better microbiological processes got firmly entrenched.

Today, prices of diosgenin are crashing down, but steroid manufacturers have also become wise with respect to relying too much on one precursor obtained from wild source which is exhaustible or at best finite, and restricted chiefly to one geographical region, Mexico.

Demand for diosgenin is expected to continue for corticosteroids, but not for sex hormones, oral contraceptives or spironolactones (diuretics) because of other precursors in the field. In fact, the development of these steroid precursors provide one of the finest examples of triumph of modern science and technology in bringing down the prices of both raw materials as well as the finished products.

POSITION IN INDIA

Dr. R.N. Chakravarti of the School of Tropical Medicine, Calcutta was the first to suggest the possibility of exploitation of diosgenin-containing Indian *Dioscorea* species at a Symposium held in Lucknow, in 1952. The following year, he discovered that *D. deltoidea* and *D. prazeri*, growing wild in the North-Western Himalayan and North-Eastern Himalayan regions respectively, contained appreciable amounts of diosgenin. The results made known in 1956 generated considerable interest in some pharmaceutical firms. Survey teams were sent to gauge the availability of the wild yams. An Indian company, Chemical, Industrial and Pharmaceutical Laboratories, India (CIPLA), entered the steroid field and began producing diosgenin and sex hormones commercially in 1961. Wyeth Laboratories followed suit by setting up a steroid plant in Bombay in 1962, and are today the largest unit in India producing steroid drugs, particularly corticoids, with diosgenin as the starting material. Subsequently CIBA-Geigy, Searle and Organon set up plants in India to manufacture sex hormones including contraceptive ingredients and anabolics. Glaxo and Merck had also started manufacturing corticosteroids in early 60's, but they used imported penultimate intermediates as starting material. Of these Glaxo has switched over to local diosgenin and subsequently to its intermediate for producing betamethasone.

In 1966-67, Central Indian Medicinal Plant Organisation (CIMPO), Lucknow of CSIR, entered the commercial production of diosgenin in its Drug Factory, Jammu, utilizing the wild *D. deltoidea* from the forests of Jammu & Kashmir state.

Sex hormones, anabolic agents, corticosteroids and oral contraceptives are being produced in India, using only diosgenin as the starting material. The use of oral contraceptives was approved in the country in the mid-sixties and the active ingredients for these are also being produced in the country, but the 'pill' has not received much favour in India as it did in the western countries.

India depended almost entirely on the wild *D. deltoidea* tubers for its diosgenin until recently. The large scale collection of the tubers has, however, resulted in depletion of forest resources and in some areas complete eradication of the wild plant. The natural regeneration of this species requires more than 7 years; hence it has been felt necessary to bring it under cultivation and also to introduce some exotic *Dioscorea* spp. for the regular and assured supplies of tubers.

Successful cultivation of *D. floribunda* and *D. composita* in Bangalore, Jammu, Goa and other parts of the country is being reported from 1973 onwards. Extraction of diosgenin from cultivated tubers along with the wild ones has already commenced. *D. deltoidea*, much valued for its purest possible source of diosgenin has now been reported to be brought under cultivation although it proved adamant in initial stages.

Considerable progress has been made in India in the R & D work on diosgenin-bearing plants and in the steroid industry. High-yielding strains of dioscoreas, particularly of *D. floribunda*, have been developed. Intense activities are currently going on to multiply the same by tissue culture technique; to improve the farm technology so as to cut down the expenses on cultivation of dioscoreas; and to improve extraction and conversion processes of diosgenin with accent on fermentation technology.

New sources of diosgenin, such as *Costus speciosus* and *Kallstroemia pubescens* are being developed for commercial exploitation. With the growing demand for steroid drugs, production of other precursors like solasodine and hecogenin is already on the stream. Emphasis is likely to shift to the recovery of phytosterols and cholesterol from industrial by-products as a sequel to the recommendations of the Symposium on "Vegetable Raw Materials for Steroid Hormones and Oral Contraceptives" held in March, 1977, at the National Botanic Gardens, Lucknow.

REFERENCES

Applezweig, N. Steroid Drugs (McGraw-Hill Book Co., Inc., New York), 1962.

Applezweig, N. Dioscorea—the pill crop (Paper presented at the Annual Meeting of the Society for Economic Botany University of Illinois at Urbana, June 13-16, 1976).

Bammi, R.K. & Randhawa, G.S. Dioscorea Improvement Project—Status Report (presented to the Joint Coordinating Committee of the Dioscorea Improvement Project of. the Indian Council of Agricultural Research, Council of Scientific & Industrial Research and the Ford Foundation, New Delhi), 1972.

Chakravarti, R.N. Steroid intermediates for the Indian steroid industry. *J. Inst. Chem. India*, 1976, 48, 151-64.

Chakravarti, R.N., Chakravarti, D.R. & Barua, A.K. Utilization of Indian dioscorea plants for preparation of cortisone. *Indian Med. Gaz.*, 1953, **88**, 1-18.

Fieser, L.F. & Fieser, M. Topics in Organic Chemistry (Reinhold Publishing Corporation, New York; Chapman & Hall, Ltd., London), 1963, 260.

Hamied, Y.K. Progress and prospects of the steroid industry in India (Lecture delivered at the Indian Institute of Science, Bangalore, March 8, 1976).

Kirk-Othmer Encyclopedia of Chemical Technology (John Wiley & Sons, Inc., New York), 2nd ed., Vol. 18, 1969, 845-46, 849.

Kreig, M.B. Green Medicine, the Search for Plants that Heal (Rand McNally & Co., New York), 1964, 255-71, 272-92.

Kunjithapadam, S. Utilization of various raw materials for the production of steroids, *in* Cultivation & Utilization of Medicinal and Aromatic Plants, edited by Atal, C.K. & Kapur, B.M. (Regional Research Laboratory, Jammu-Tawi), 1977, 9-20.

Taylor, N. Plant Drugs that Changed the World (George Allen & Unwin Ltd., London), 1964, 228-58.

CHAPTER 2

SOURCES OF DIOSGENIN

The rhizomes of plants belonging to the genus *Dioscorea* Linn., commonly known as yams, are the main sources of diosgenin, though this sapogenin can also be obtained from some other genera (1,2). Dioscorea tubers from wild plants are being commercially exploited for their sapogenin in Mexico, India, China and Guatemala and some species have been brought under cultivation for this purpose. Diosgenin can also be obtained synthetically, but synthetic route has not been found economical (3a, 4).

The natural sources of diosgenin fall under three broad groups: (a) Wild dioscoreas, (b) Cultivated dioscoreas, and (c) Plants belonging to other genera, such as *Balanites* Delile, *Costus* Linn., *Kallstroemia* Scop., *Tribulus* Linn., and *Trigonella* Linn.

Diosgenin-containing plants are listed in Appendix I.

DIOSCOREAS

Dioscorea Linn. (*Dioscoreaceae*) is a genus of about 600 species of annual twining herbs, except the dwarf dioscoreas of the section *Borderea*, distributed throughout the moist tropics of the world, extending into the warm temperate regions (2). About 50 species are found in India (3b, 5a). A good number of these species occur in a wild state in almost all parts of India, except the dry north-western region; a few species such as *D. alata* and *D. esculenta*, are cultivated for their starchy edible tubers, called the true yams (3a).

Morphology—A typical member of the genus *Dioscorea* is a dioecious plant consisting of a rhizome, or tuber as it is called in trade, or a group of rhizomes from which the roots and the aerial vines emerge annually during the growing season. The diosgenin-containing rhizomes of the wild plants are perennial organs, slowly increasing in size with age and usually free from all attacks of fungi and chewing pests (2).

The plants are of two types: those with stems twining to the right, and those with stems twining to the left. Though *Dioscorea* is a monocotyledonous* genus, the leaves of its members are cordate and acuminate with 5-9 basal nerves, which are more typical of dicots. The

---

*Lawton and Lawton have shown that the embryos of some *Dioscorea* species may have two cotyledons. This may affect the fundamental classification of the genus [*Proc. Linn. Soc.*, 1967, **178**(2), 153].

fruit is a trilocular, 3-winged capsule opening by three valves. The seeds are small, light, flattened and winged. Natural regeneration is through seeds which easily get dispersed by wind. Vegetative propagation is done by rhizome pieces and cuttings of the leaves and stems (2, 3a). Propagation through single node leaf cuttings and other tissue culture techniques is also being tried at some places.

The cultivated yams, which are grown as garden crops or subordinate crops with ginger, turmeric, brinjals, sweet potato and maize, are, however, propagated by tubertops and to a much lesser extent by bubils borne on the aerial stems (5a).

WILD DIOSCOREAS

There are several species of *Dioscorea* which are known to contain diosgenin (Table 2.1), but not all are commercially exploitable (4, 1). A diosgenin content of around 3 per cent on moisture-free basis, is normally the accepted minimum level for commercial utilization of the rhizomes (6ab). In Mexico and Guatemala, rhizomes of *D. composita*, *D. floribunda*,

TABLE 2.1—IMPORTANT DIOSGENIN-CONTAINING DIOSCOREA SPP. AND THEIR GEOGRAPHICAL SOURCES[1]

Species	Geographical source
D. althaeoides R. Knuth[2]	China
†*D. balcanica* Kosanin	Europe
D. belizensis Lundell	Central America
**D. composita* Hemsl.	Mexico, Guatemala
D. deltoidea Wall.	India, Pakistan, Nepal, China
—var. *orbiculata* Prain & Burk.[2]	China
**D. floribunda* Mart. & Gal.	Mexico, Central America
†*D. friedrichsthalii* R. Knuth	Central America
D. glauca Muhl.	United States
†*D. hondurensis* R. Knuth	Mexico, Central America
**D. mexicana* Guill.	Mexico, Central America
D. nigrescens R. Knuth[2]	China
**D. panthaica* Prain & Burk.[2]	China
**D. prazeri* Prain & Burk.	India
**D. spiculiflora* Hemsl.	Mexico, Central America
†*D. sylvatica* Eklon	South Africa
D. villosa Linn.	United States
**D. zingiberensis* C.H. Wright[2]	China

[1] International Trade Centre UNCTAD/GATT Markets for Selected Medicinal Plants and their Derivatives. Geneva, 1974. Chapter IV—Dioscorea and Diosgenin, 68.

[2] Gerasimenko & Tropova, *Rastit. Resur.*, 1971, 7, 192-200.

*Used commercially.

†Potential sources.

and to a lesser extent *D. spiculiflora* and *D. mexicana* are collected for extraction purposes. In China, rhizomes of *D. zingiberensis*, *D. deltoidea* and *D. panthaica* are the sources of diosgenin (7). The aerial parts of *Dioscorea* spp. have also been studied but the diosgenin content is too low to be of economic importance (6a).

In India, *D. deltoidea* occurring in the north-western Himalayas, is the major source of diosgenin (1, 7, 8). Collections of the rhizomes of *D. prazeri*, occurring in the moister parts of the central and eastern Himalayas have also been recorded (9, 6b). According to steroid drug manufacturers, it is an inferior source of diosgenin (7). Of all the dioscoreas, *D. deltoidea* is the only known source which provides purest possible diosgenin and is thus highly valued by the manufacturers for this purpose (8).

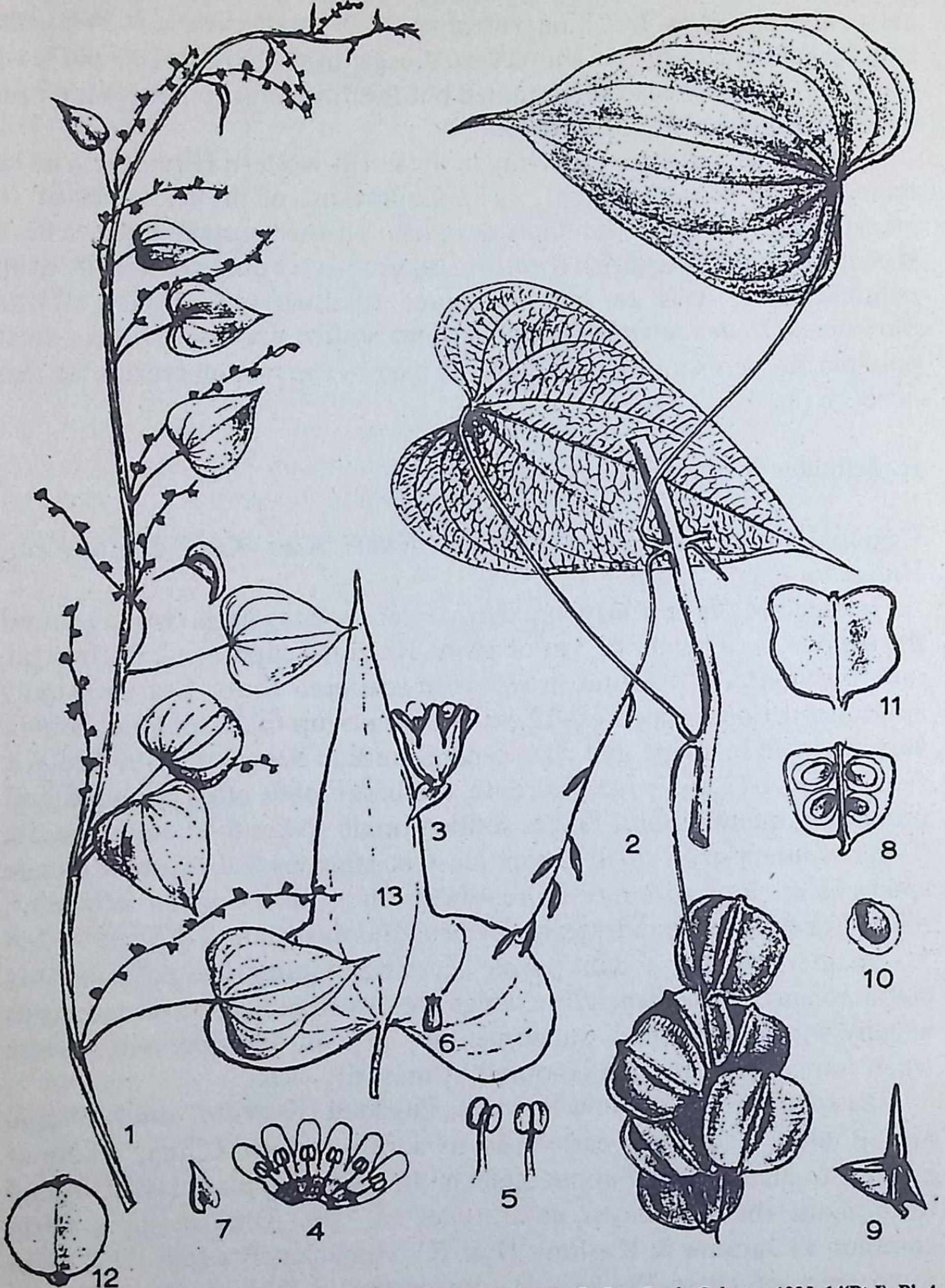
D. deltoidea Wall. (Figs. 2.1 & 2.2)

Regional names—Jammu & Kashmir: *Kildri*, *Kins*, *Kithi*, *Krish*, *Krits*; Himachal Pradesh: *Shingli*.

It is a glabrous or nearly so, extensive, left-twining herb, twining round the support to a height of 5 m or more. Rhizome superficial, horizontal, tuberous, digitate, chestnut brown with scattered roots. Leaves usually alternate, petiolate; petiole 5-12 cm, sometimes up to 25 cm long; lamina very variable in shape and size, general outline being ovate-lanceolate, 5-15 cm × 4-12 cm, widely cordate, the basal lobes often rounded and projecting, membranous. Spikes solitary; male spikes 8-40 cm in length; flowers solitary or in small distant clusters, stamens 6, didymous; female spikes 15 cm long with 6 or more flowers on a spike. Capsule orbicular, deltoid or obtusely quadrate, rarely broadly obcordate, 2-2.5 cm × 2.5-3.5 cm, green with abundant purple flecks when immature, reddish-stray coloured and reflexed when ripe, wings broadly rounded, 4-6 seeded; seeds usually winged all round, but sometimes only on one side, milky white when immature, but fawn coloured at maturity (10).

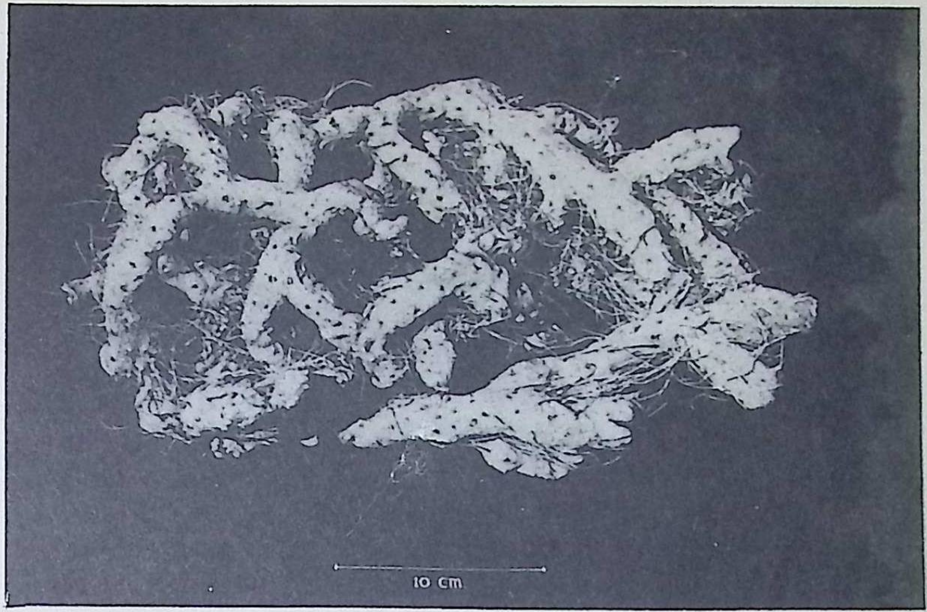
Distribution—It is found in India, Pakistan (Kurrum valley), Nepal and Bhutan, extending eastwards to south-western China, where it ascends to an altitude of about 3,600 m. In India, the plant is distributed throughout the Himalayas at altitudes of 1,000-3,000 m. It is fairly common in Jammu & Kashmir (J & K), Himachal Pradesh (H.P.) and some parts of Uttar Pradesh. It also occurs in Sikkim, West Bengal, Arunachal Pradesh and Meghalaya (10, 11, 12a, 13, 14).

Jammu & Kashmir—*D. deltoidea* is fairly widespread in Bhadarwah, Kishtawar and Doda forest divisions of Jammu, and in Kashmir, Sidh and Sangat forest divisions of Kashmir. Qualitatively, the stock growing in Bhadarwah and Kashmir forest divisions is stated to be the best in India.



Prain & Burkill, Ann. R. Bot. Gard., Calcutta, 1935, **14**(Pt I). Pl. 4

Fig. 2.1—*Dioscorea deltoidea*: 1, A branch with male flowers; 2, A branch with capsules; 3, A male flower; 4, The same opened, diagrammatic; 5, Stamens; 6, The gynoecium of a male flower; 7, A bract; 8, L.S. Capsule; 9, T.S. Capsule; 10, A seed; 11, A capsule of unusual shape; 12, A capsule of var. *orbiculata*; 13, An unusual leaf from a specimen from Dalhousie.



RRL, Jammu

Fig. 2.2—Dried rhizome of wild *Dioscorea deltoidea.*

In other areas, the vine is encountered in interrupted patches in Reasi, Punch, Ramban and Jhelum valley forest divisions (10, 12c).

Himachal Pradesh—The plant is more widely distributed in catchment areas of the rivers Ravi and Beas in Chamba and Kulu valleys respectively. It is also fairly common in upland regions of Kangra valley and Kinnaur. The raw material from Palter area is comparable to that available in Kashmir (10, 12c).

Uttar Pradesh—The plant is gregarious in upper reaches of Tons and Jamuna valleys in Jaunsar, dry temperate regions of Uttar Kashi and Chamoli in Garhwal, and Pithoragarh in Kumaun Hills. In other areas it is encountered only occasionally as a weed in oak-coniferous forests (10, 12c).

Sikkim—The plant occurs in Chungthang-Singhik region of Sikkim (13).

West Bengal—*D. deltoidea* is met with in Darjeeling area (10).

Arunachal Pradesh—*D. deltoidea* is found, though rarely, in Jigon to Rupa region (Kameng) (13).

Meghalaya—The species occurs in Khasi Hills (13).

Diosgenin content—The colour of the flesh of the tuber varies from pure white to deep yellow with intermediate hues of pink, blue, and purple (3b). In general, tubers with white flesh are said to contain more diosgenin than the ones having yellow flesh, but it is not always so (7). Tubers from Jammu & Kashmir generally have white flesh and are supposed to be of

TABLE 2.2—DIOSGENIN CONTENT OF THE TUBERS FROM DIFFERENT AREAS*

Area	Altitude (metres)	Tuber size (on dry wt basis) g	Diosgenin content %
J & K State			
Bhadarwah	—	52	4.8-8.0
Katra	1,500-1,800	45	4.6-4.8
Gulmarg	2,000-2,500	28	4.6
Pahalgam	2,000-2,500	25	3.8
Batota	1,500-2,000	18	4.3
Sanasar	2,000-2,400	15	3.8
Punch	1,800-2,000	12	trace
Himachal Pradesh			
Kulu	1,800-2,400	42	4.3-5.8

*Abrol & Kapoor, *Bull. Reg. Res. Lab., Jammu*, 1962-63, 1, 30-31.

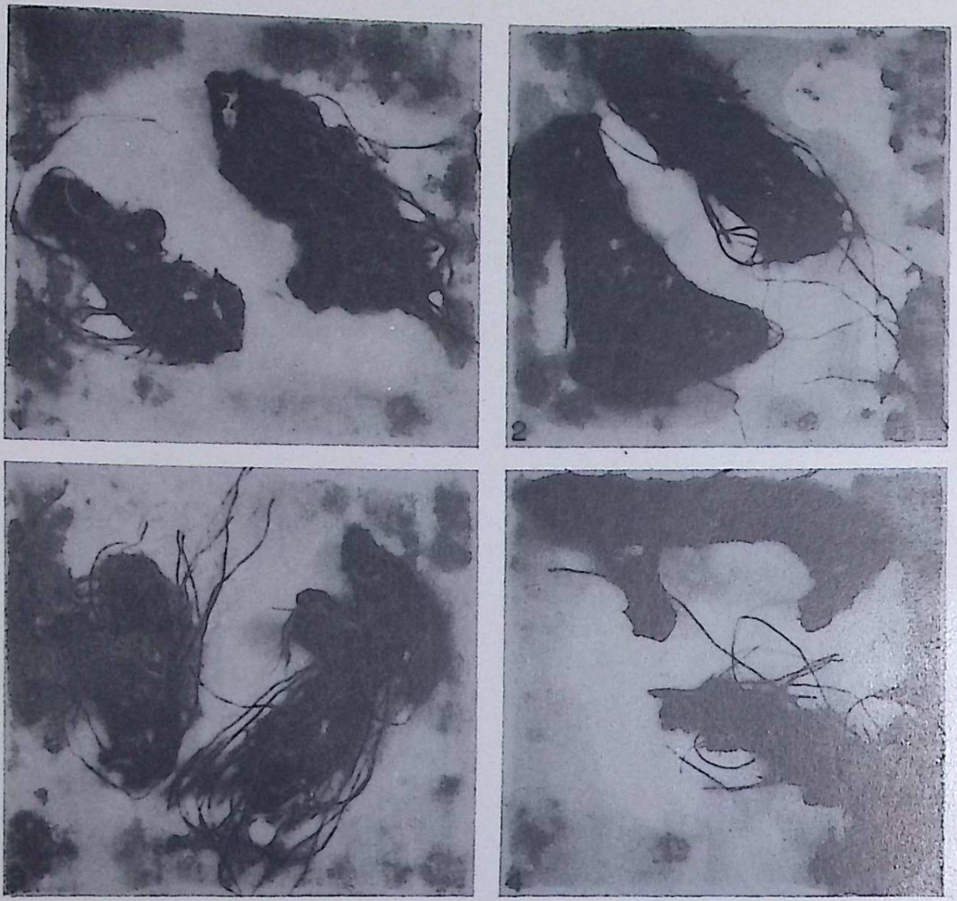
the best quality (3b), though the genin obtained from yellow tubers from some parts of Himachal Pradesh, according to a manufacturer of diosgenin is in no way inferior (7).

Tubers from different areas are reported to show wide variation in their diosgenin content, ranging from traces to 10 per cent (4, 3b). Diosgenin content increases in the tubers, to a limited extent, with the age of the plant, but there is no correlation between the girth of the tuber and the diosgenin content, as the age of the rhizome is determined by the intensity of proliferation of the fingers and not just by girth (11, 3b, 12c). Table 2.2 gives the diosgenin content of the tubers collected from various places.

Availability of the tubers—The total quantity of reserve stock of tubers of *D. deltoidea* in India, calculated on the basis of 1970 figures, has been estimated at about 12,000 to 15,000 tonnes. Field investigations in a number of localities have indicated the possibility of a fair re-stocking over a period of 10 years, provided ample stock of rhizomes is left in the forest area for regeneration after the collection of the rhizomes. It is estimated that the annual supplies of the rhizomes from forests can be increased to 1200 tonnes without much depletion of the natural stock, enabling an annual production of 35 to 40 tonnes of diosgenin, or even more, depending on the diosgenin content of the available material (12a).

Yearwise collection of tubers of wild *D. deltoidea* from Jammu & Kashmir, Himachal Pradesh and Uttar Pradesh is given in Table 2.3. It is, however, not possible to vouch for the accuracy of these data, since different sources give different yearwise figures, and no information is

Pl I



PIECES OF DRIED RHIZOMES OF WILD DIOSCOREA DELTOIDEA FROM DIFFERENT REGIONS

(pieces are 8 to 10 cm long)

1, Kashmir	2, Kulu (H.P.)
3, Chamba (H.P.)	4, Simla (H.P.)

TABLE 2.3—COLLECTION OF D. DELTOIDEA TUBERS FROM VARIOUS STATES[1]

(Qty in tonnes on dry wt basis)

Year	Jammu & Kashmir	Himachal Pradesh	Uttar Pradesh	Total
1960-61	S.T.	346.067	Nil	346.067
1961-62	73.640	49.508	2.56	125.708
1962-63	0.130	804.000	S.T.	804.130
1963-64	56.620	775.000	S.T.	831.620
1964-65	34.080	674.200	S.T.	708.280
1965-66	110.200	398.086	55.00	563.286
1966-67	88.647	586.012	23.00	697.659
1967-68	41.308	686.000[2]	S.T.	727.308
1968-69	49.738	536.000	Nil	585.738
1969-70	72.100	539.764	Nil	611.864
1970-71	130.392	470.100[2]	Nil	600.492
1971-72	91.800	395.050	Nil	486.850
1972-73	28.067[3]	683.800[2]	Nil	711.867
1973-74	36.400[3]	162.400[2]	N.A.	198.800
1974-75	54.506[3]	N.A.	N.A.	54.506
1975-76	93.232[3]	427.900[4]	N.A.	521.132

[1] Sarin, *Indian Drugs*, 1974, **11**(10, 15-17; [2] Tyagi, *et al.*, Proc. Second Workshop of AICIP held at Anand, 1976, 214-18; [3] Information from CIMPO, Lucknow; [4] Information from the Chief Conservator of Forests, Himachal Pradesh, Simla.

S.T.—Small transactions only. N.A.—Information not available.

available, from the private agencies involved in the collection of tubers, particularly in Himachal Pradesh areas (12b, 15ab, 16, 17a, 18a). There are thus no records of authentic transactions. It is estimated that the annual production of diosgenin is 30 tonnes; on the assumption of 3 per cent recovery of diosgenin, the total quantity of tubers processed come to about 1,000 tonnes per annum.

Need for conservation—The supplies of *D. deltoidea* come from wild sources, and ruthless and indiscriminate exploitation of the plant without proper assessment of the resources and adequate regeneration measures is a serious problem, which has resulted in depletion of the natural stocks. The areas of occurrence of this species have been surveyed a number of times since 1962, and these surveys indicate that although the plant was found in abundance during the earlier trips, it now seems to be getting rarer (3a). With the depletion of natural resources, the price of the tubers has shot up over the years from Re 1 per kg in 1961 to Rs. 11 per kg in 1976, thereby causing a proportionate increase in the price of diosgenin (95% purity) from Rs 100 to Rs 1306 per kg in 1976 (12b, 7). The prices are now showing a downward trend (Rs 6-7/kg) due to the availability of cultivated tubers (8).

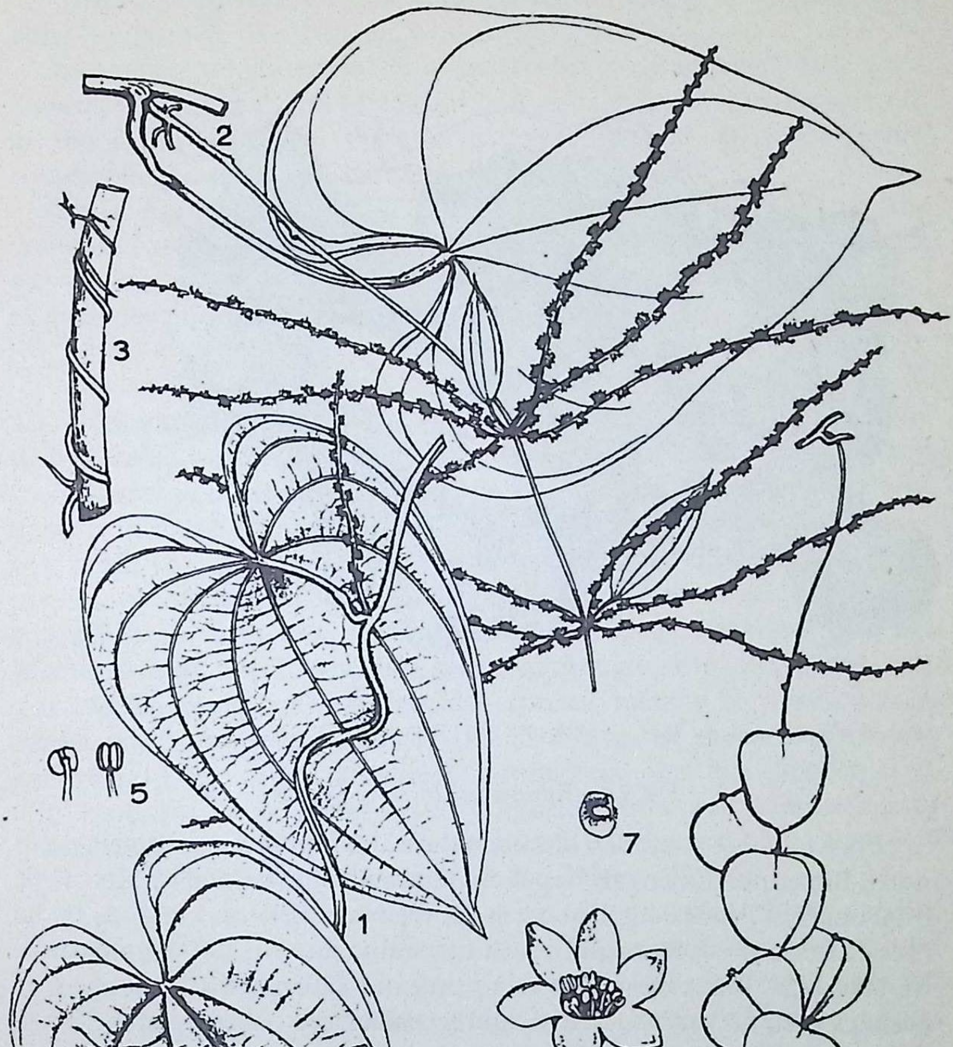
Fearing exhaustion of the stocks, the J & K Government brought this item under '*Kuth Act*' for strict control over collection, extraction, transport, and trading of the drug outside the state. Also, collection and trade of the tubers was taken over by the Forest Department of the state itself (15a). But in Himachal Pradesh, owing to laxity in supervision and easy procedure for collection of the minor forest products on payment of collection/export fee, the exploitation of the plant continues unabated (12a, 15b).

The following measures have been suggested to prevent depredation of *D. deltoidea:* (i) A practice of 7-year rotational extraction of rhizomes from various forest compartments may be adopted; (ii) re-seeding of forest areas by tubers or by seeds is essential and should be regularly done; the tuber pieces should not normally be less than 60 g in weight and should be planted in the rainy season, 2-3 rhizome pieces in each pit, preferably with sprouted tips, followed by closing of each worked out compartment for a minimum period of 7 years; (iii) the seed obtained from regions of high diosgenin content should be broadcast over a wide area when the conditions are ideal for germination, so that the seedlings may not dry up; and (iv) high-yielding clones from Verinag, Bhadarwah and Tial forest areas in Jammu & Kashmir should be conserved for propagation (3a, 12c).

D. prazeri Prain & Burk. (Figs. 2.3 & 2.4)
syn. *D. clarkei* Prain & Burk.; *D. deltoidea* Wall. var. *sikkimensis* Prain; *D. sikkimensis* Prain & Burk.

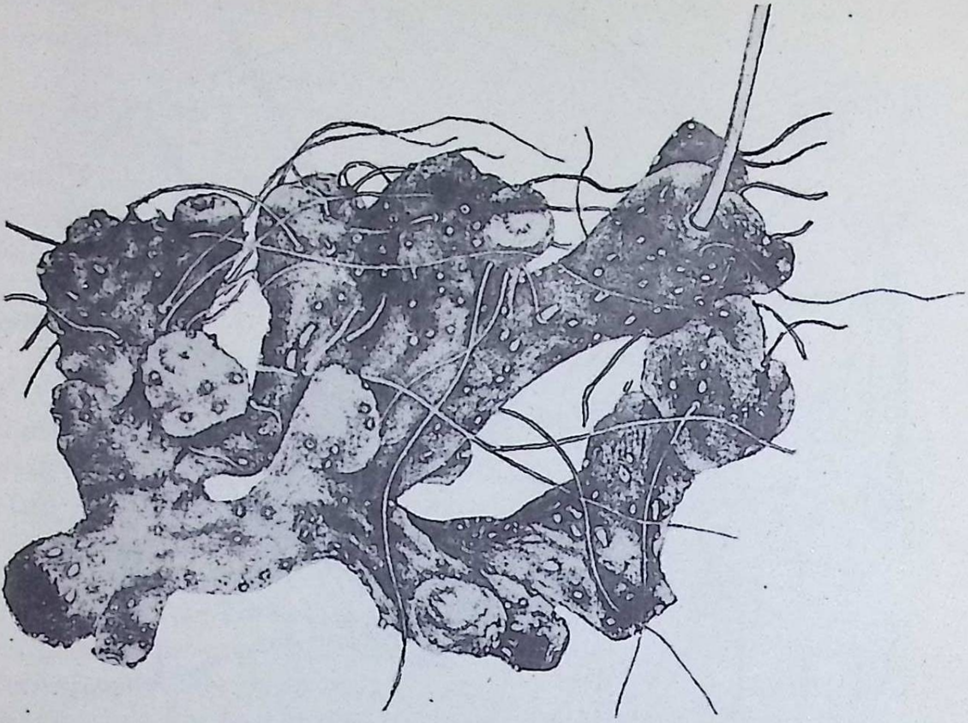
Regional names—West Bengal and Eastern Himalayas: *Kukur torul*; Lepchas: *Kencheong* (19, 20).

It is left-twining herb, ascending to a height of 4 m or more. Rhizome very poisonous, superficial, stout, horizontally creeping, freely branched; branches about 10 cm × 1.5-2 cm, grey brown-blackish, flesh white or creamy. Stem terete or faintly ridged, sometimes purplish-brown speckled. Leaves alternate, rarely opposite on the lower nodes; petiole 4-8 cm; lamina ovate, usually 12 cm × 10-16 cm from petiole to acumen, sometimes up to 20 cm × 30 cm, cordate, acuminate, basal lobes divergent, nerves usually 7, sometimes 9-11. Male flowering axes 2 or more together, rarely solitary axillary, slightly winged and twisted, up to 20 cm long, transitional stages approaching a terminal panicle are found; male flowers sessile, 1-3 together; female flowering axis solitary, axillary, 12 cm or more in length, bearing up to 20 flowers; capsules sessile, about 8 in a spike, reflexed on maturity, chestnut brown on ripening, base obtuse, apex cordate; wings 2.2-2.5 × 1.2-1.5 cm; seeds with 5-7 mm broad wing all round (10, 14).



Prain & Burkill, Ann. R. Bot. Gard. Calcutta, 1936, 14(Pt I), Pl. 5

Fig. 2.3—*Dioscorea prazeri*: 1 & 2, Branches with male flowers; 3, The twining apex of (1); 4, A male flower; 5, Stamens; 6, Capsules from Bhamo, Burma; 7, A seed slightly immature; 8, A capsule; 9, A mature seed.



Prain & Burkill, Ann. R. Bot. Gard., Calcutta, 1936, 14(Pt I), Pl. 6

Fig. 2.4—Rhizome of *Dioscorea prazeri.*

Distribution—*D. prazeri* occurs in the wetter parts of the Himalayas in north Bihar, north Bengal, Nepal, Sikkim, and Bhutan, and in Abor Hills of Arunachal Pradesh. It also occurs in the Naga Hills and extends to the hills of Burma and Siam and south-eastwards into the northern parts of Malaysia (5a, 10). It ascends to an altitude of 1,220 m in the Himalayas, or perhaps even higher, to 1,525 m, and has also been collected in the Naga Hills at an elevation of 1,690 m (10).

It seems to prefer well drained soils; high banks of the streams suit it well (10).

Sikkim—It occurs in *Shorea robusta* forests (10).

West Bengal—It is found mostly in Darjeeling terai and Jalpaiguri and Malda districts (10).

Bihar—Champaran district (10).

Arunachal Pradesh—It occurs in Siang (14), and Tawang regions (21). The tubers collected from Lumla, Sakti, Gispu, Bongleng, and Khepbara in Tawang region, according to information available on the basis of a 1976 botanical exploration, contain 3.5 per cent diosgenin.

Nagaland—It occurs in the Naga Hills, but no collection on a commercial scale is done in this state (15c).

Mizoram—It occurs in the Lunglai district and it is estimated that only 3-4 quintals of tubers are available (15d).

Rhizomes are stated to be available on a commercial scale from Sikkim, West Bengal, and Mizoram (7). A visual assessment indicates that in the Tawang region, there is a large stock of *D. prazeri* with commercially exploitable diosgenin content (21).

Small scale extraction is possibly being done, but neither the concerned agency nor the amount extracted is known. According to some steroid drug manufacturers, *D. prazeri* affords very impure diosgenin and as such does not find favour with them (7, 8).

DIOSCOREAS UNDER CULTIVATION

The wild sources of *D. deltoidea* are dwindling in India, and supplies of wild material, with optimum diosgenin content, are becoming more and more scarce and expensive. Cultivation of suitable species of *Dioscorea* appears to be the solution to maintain regular supplies of raw material for this genin at reasonable prices. Several investigators have studied the possibility of bringing diosgenin containing *Dioscorea* spp. under cultivation in both India and abroad. Prominent amongst these are Martin & Gaskins (22), who have reviewed the cultivation of the four most suitable diosgenin-bearing American species, namely *D. floribunda*, *D. composita*, *D. friedrichsthalii* and *D. spiculiflora*. Of these, the first two species have been found suitable for commercial planting. Blunden *et al.* (6b) have reviewed all aspects of hybridization and cultivation of *Dioscorea* species, wherever they are grown. Cultivation of dioscoreas in India has been studied by Bammi & Randhawa (3a).

D. deltoidea, though exacting in its climatic and soil requirements, has shown some promise as a cultivated source of diosgenin in the U.K. and U.S.S.R. (23, 4). In spite of its slow cycle, trials of this species at CIMPO Farms at Manasbal and Yarikha (J & K) have given encouraging results (24, 25). In other parts of India, it has shown an adaptation breakdown. *D. prazeri*, another wild species, which is under experimental cultivation at the foothills of the eastern Himalayas in Darjeeling terai, appears to be less promising both in terms of tubers as well as diosgenin yields. As such, the emphasis in India has shifted to cultivation of Central American species, namely, *D. floribunda* and *D. composita* (3a).

Commercial cultivation of *D. composita* and *D. floribunda* is being carried out around Bangalore and in the Union territory of Goa by Wyeth Laboratories (India), CIPLA, Searle (India) and CIBA-Geigy of India (7). CIPLA has a programme of setting up plantations in Coorg and in the Andamans (26). The Indian Institute of Horticultural Research (IIHR), Bangalore, the Indian Agricultural Research Institute (IARI), New Delhi, and the National Botanic Garden (NBG), Lucknow grow *D. floribunda*

TABLE 2.4—AREA UNDER CULTIVATION OF DIOSCOREA SPP.
(in hectares)

	*D. floribunda**	*D. composita**	*D. prazeri**	*D. deltoidea*†
Bangalore	40.90	33.60	—	—
Goa	6.00	6.00	—	—
Dibrugarh	1.20	—	—	—
Ootacamund	—	58.00	—	—
Yarikh & Manasbal	—	—	—	5
Darjeeling district	—	1.50	25	—
Total	48.10	99.10	25	5

*Information through personal communication; figures are for the year 1976.

†Information from CIMPO, Lucknow.

Note: These figures do not include area under cultivation by IIHR, Bangalore, NBG, Lucknow and IARI, New Delhi for providing planting materials to farmers for cultivation.

with high-yielding strains for providing tubers to commercial growers (3a, 7). *D. composita* is grown by Cinchona Department, Tamilnadu, in Anaimalai Hills for diosgenin extraction by the department (27). Its cultivation has also been taken up in the foothills of Darjeeling district, by the Directorate of Cinchona & Other Medicinal Plants, Govt. of West Bengal. About 100 tonnes of dried tubers, derived from the 3-year old crops of these two species are, being processed by one of the multinational drug companies in India since 1975 (7). Among the cultivated species, *D. floribunda* has so far given the best results, particularly under Bangalore conditions. Table 2.4 gives the area under cultivation of *Dioscorea* spp. at different places in India.

Salient features of *Dioscorea* species and other plants being used or under investigation as source materials for diosgenin in India are summarized in Table 2.5.

Agronomy

A general account of the cultivation of *Dioscorea* spp. is given here. Other details are described under the individual species.

Propagation—Dioscoreas can be propagated from seeds, from leaf or stem cuttings, or from pieces of rhizomes. According to Martin and Gaskins, propagation from seeds is considered the most reliable method under Puerto Rican conditions, though asexual propagation may be used profitably under certain conditions (22). In India, however, propagation is mostly done by rhizome pieces, particularly in the case of selected clones.

TABLE 2.5—SALIENT FEATURES OF PLANTS BEING USED OR UNDER INVESTIGATION AS SOURCES OF DIOSGENIN

Botanical source	Plant part	Diosgenin content (%, dry wt basis)	Habitat and Cycle
Dioscorea deltoidea[1]	Tubers	2-4.8	Growing wild in sub-Himalayan region. Under cultivation trials in Kashmir. Cycle: 3-7 years.
D. prazeri[3]	Tubers	up to 3.5 fairly pure	North-eastern sub-Himalayan region. Can be cultivated in the region. Cycle: 3 years
D. floribunda[1]	Tubers	2-5	Central American species. Grows well in peninsular and Northern India. Low altitude locations. Cycle: 1-3 years.
D. composita[1]	Tubers	2-5	Central American species. Grows well in peninsular India in particular. Cycle: 3-4 years.
Costus speciosus[2]	Rhizomes	up to 2.5	Grows wild all over the hilly region of North and West. Cultivation tried in North-west. Cycle: 1-1¼ year.
Kallstroemia pubescens[4]	Whole plant leaves	0.9-1.7; 1.8-1.9	Tropical American weed naturalized in West Bengal around Howrah and Burdwan. Cycle: 6 months.
Trigonella foenum graecum[5,6]	Seeds	0.7-1.7*	High sapogenin-yielding cultivar under experimental cultivation at Bangalore. Cycle: 3-5 months.
Balanites aegyptiaca†	Fruit wall seeds	1.5*,† 0.6*,†	Perennial plant found mostly in semi-arid zones.

[1] Kunjithapadam, *in* Cultivation & Utilization of Aromatic & Medicinal Plants, 1977, 10; [2] Sarin *et al.*, ibid., 35; [3] Information from Dr G. Thyagarajan, Director, RRL, Jorhat; [4] Chakravarti *et al.*, *J. Inst. Chem. India*, 1976, **48**, 170-73; [5] Blunden *et al.*, *Trop. Sci.*, 1975, **17**, 139-54; [6] Information from Dr Y.K. Hamied, Managing Director, CIPLA, Bombay; [7] Ghanim & Jayaraman, Paper presented at Symp. on Arid Zone Res. Develop., held at Jodhpur on Feb. 14-18, 1978.

*Includes yamogenin; †Fresh wt basis.

Soil—Dioscorea species in general are tolerant to a variety of soil and climatic conditions (22); but *D. deltoidea* is a very fastidious plant and, as already mentioned, it has exhibited adaptation break downs in cultivation experiments at Bangalore and Lucknow (3b). A study of soil profile under *D. deltoidea*, conducted at Kashmir and Kulu, indicated a preference to slightly acid soils of sandy-loam texture. It thrived in alluvial soils and remained unaffected of the gravel in the lower strata (12c). Cultivation of *Dioscorea* spp. gives best results in well-drained loamy soils in locations with prolonged rainy season. Though the vines show tolerance to drought, supplemental irrigation is advisable during the dry spells. Tubers in sandy soils tend to be small, but they can easily be harvested and cleaned. Extremely heavy clayey soils restrict plant growth and impede harvesting later. Dioscoreas tolerate a fairly wide variation in *p*H of the soil. Very acid soils should be avoided as far as possible, and should be limed before use (22).

Soil types and methods adopted for soil preparation affect the shape of the rhizomes and ease of harvesting. In light soils, the rhizomes are longer, thinner, and deeper as compared with the ones in heavy soils. A hard subsoil deflects the tubers and leads to horizontal growth. Such subsoil below 15-20 cm, however, does not affect the shallow-rooted species like *D. deltoidea* (22, 12c).

On well-drained soils, ploughing and harrowing, sufficient enough to provide a loose bed *c.* 20 cm deep (see under cultivation of individual species) is needed. In poorly drained soils, raised beds are used, as they not only increase soil aeration, but also facilitate harvesting. Planting can be done by hand or by vegetable planting machines.

Supports—The dioscorea vines must be properly supported for their optimum growth. The characteristics desirable for any support system are: (i) low cost, (ii) a support height of 4.5-5.5 m, (iii) individual support for each vine, and (iv) desirability of the support lasting for 3-4 years. Post-and-wire and stake supports are satisfactory; so are pillar-and-trellis supports, where each plant is supported by twines pegged to the ground and tied to the overhead wire. Light stakes, such as small bamboo culms or dried stems of *Sesbania* sp. can also be employed for this purpose. Post-and-wire and stake support is more expensive than bamboo sticks, but is cheaper than pillar-wire system (6b). Experiments conducted on *D. composita* have initially shown that non-living supports lead to increase in both tuber weight and yield of the sapogenin (22).

Weeding—Dioscoreas are slow in their growth during the first year and, therefore, have to stand heavy competition with the weeds; but mature dioscoreas are vigorous and aggressive and are capable of inhibiting weed growth due to their excessive shade and competition for nutrients (22). In Bangalore, nut grass (*Cyperus rotundus* Linn.) is the predominant weed in *D. floribunda* fields which can be controlled by Karmex, Tok, Simazine, Atrazine, or Telwar (3 kg/ha). Bomacil, Tapapon, Sinabar, and Herban proved phytotoxic to dioscoreas (3a). Conventional cultivation techniques to control weed should be supplemented with hand-weeding (22).

Fertilizers—The choice and requirement of fertilizers depend on the fertility of the soil. A heavy dose of organic matter is necessary for growing thick and fleshy tubers. If nitrogen (high requirement, up to 336 kg/ha), phosphorus (moderate requirement), and potash (moderate requirement) are deficient, lower yields are obtained.

Pests and diseases—The sapogenin-bearing species of *Dioscorea* are relatively free from attacks of diseases and pests, but the ones that attack are sufficiently debilitating to require regular and careful inspection of the crop. Various species of aphids, cotton aphid (*Aphis gossypii*) in particular and red spiders are the two pests recorded (22, 28). Aphids feed on young stems and leaves, and stunt their growth. They are also responsible for the transmission of the green-banding virus disease. The disease is very serious and causes chlorotic bleaching of interveinal areas of the leaves, giving alternate dark green and light green bands (hence the name of the virus). Plants infected with this virus are seriously weakened, their top growth is restricted, and tuber growth is drastically curtailed. The diseased plants often die in drought and other adverse conditions. This is particularly true of *D. floribunda* and *D. friedrichsthalii.* Leaf mosaic, leaf flecking, and dwarfing of the leaf and stem are frequently associated with green banding. Green-banding virus can best be controlled by prompt eradication of infected plants and by aphid control. Leaf hoppers and red spiders may also act as vectors (22). Satisfactory control of the green-banding virus in field plantings of *D. floribunda* and *D. composita* can be achieved with foliar sprays of Demeton, at 20 ml of the concentrate in 4.54 litres (1 gallon) of water, applied every three weeks (29).

Red spiders attack and feed on the undersides of the leaves. Their heavy infestation forces the plants into premature dormancy during the drying season (22). Mites have also been recorded on the vines. Akar or Kelthane (0.5%) has proved effective against mites (27). Prophylactic sprays of the insecticidal, Rogor, are beneficial against leaf-eating insects (3a). One or two sprays of Metasystox (0.1%) are also effective (17b).

The tubers are frequently attacked by snails and sludge, and grubs of large beetles. Poison baits can be used for their control. Nematodes attack the tubers and roots. *Meloidogyne incognita* (Kofoid & White) was constantly found to be the main species attacking *D. floribunda*, *D. composita* and *D. spiculiflora* in Guatemala. In light soils they cause severe damage; the affected plants are stunted and chlorotic (30 ab). Hot water treatment of the tubers at 45° for 30 minutes before planting gives a good control without affecting tuber vaibility (31a).

A fungus *Helminthosporium* sp., causing leaf spot, has been sighted in Guatemala and occasionally in Puerto Rico. Young seedlings at first leaf stage are particularly affected. Fungicidal applications twice weekly to the younger seedlings controls the disease satisfactorily (31b).

No major diseases or pests have been observed on *D. floribunda* and *D. composita* grown at Bangalore and in the Union Territory of Goa (7), but tuber rot of planting pieces was quite severe and had to be prevented by pretreatment with an anti-fungal compound like Benlate (3b).

D. composita grown in the Anaimalai Hills in Tamil Nadu is affected by mites and cutworms. Kelthane (0.5%) and aldrin dust (5%) are effective to curb these pests. Of the fungal diseases, those caused by species of *Cercospora* and *Remularia*, and a late blight due to *Phytophthora infestans* in Tamil Nadu, are important. Dithane 278 and copper fungicides (0.3%) control these diseases (27).

No serious pests and diseases, except spiders and mites have been recorded on *D. deltoidea*. These can be controlled by spray of Thiodan (0.3%). Urea (10%) can be mixed with fungicide and sprayed (25).

Harvesting—For commercial production, 3-year old tubers are generally preferred (22,7). *D. floribunda* tubers, however, can be profitably harvested even after a year or so, though 2- and 3-year old crops give better yield. Also, the diosgenin content increases with age to a limited extent (3a, 24, 25, 7).

Although tubers can be harvested at any time of the year, harvesting in the dry winter season is recommended, since during that period their genin content is relatively stable and they can be sun-dried to a safe moisture content (22).

The vines as well as their supports should be removed just before harvesting, and the field should be cleaned before the tubers are dug. After harvesting the tubers should be washed to remove excessive soil and sun-dried partially if they are not to be processed immediately (22).

To get regular supply of *Dioscorea* tubers every year, say from 3-year old crops, planting and harvesting should be done by rotation in different sectors. For example, if 30 hectares of land is available, 10 hectares of land is brought under cultivation every year. By the end of 3 years, the first 10 hectares of crop will be ready for harvesting and the next year the second 10 hectares will be ready and so on (7).

Seed production—*Dioscorea* species are dioecious and bear seed after cross pollination. It has been found in *D. floribunda* that there is no seed-set unless the male and female plants are in extremely close physical proximity. When they were intertwined or they are hand pollinated, good seed-set is obtained. Under normal field conditions, the vines become completely entangled and, therefore, set seeds freely during the second and third years (22, 3a).

D. floribunda Mart. & Gal.

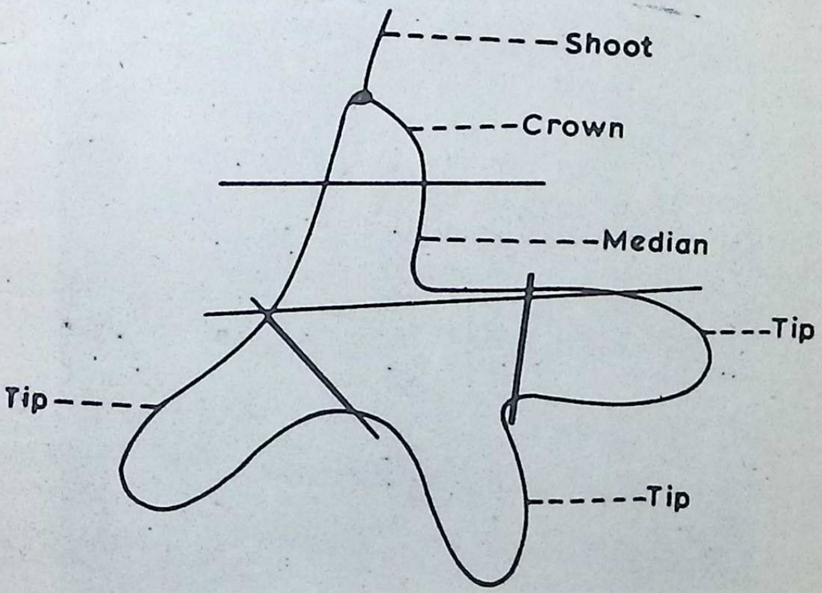
It is a herbaceous, glabrous, left-twining vine with thick tubers, branched and deep rooted up to 30 cm depth. Stems slender or stout, sinistrose.

Leaves alternate, broadly ovate or triangular ovate, shallowly or deeply cordate, coriaceous, 9-nerved; petioles, 5-7 cm long, rather thick and firm. Male flowers solitary, rarely in pairs; female flowers with diverricate stigma, bifid at the apex. Capsule obovate; seeds winged all round (32).

Results obtained from the trials at Bangalore indicate that *D. floribunda* strain *FB* (*C*-1), introduced from Central America, is suitable for commercial cultivation. The plants of this strain are vigorous, fast growing and relatively free from diseases and pests, and have moderate to high content of diosgenin (3a).

Pusa-1, a new selection from the germplasm of *D. floribunda*, is doing well at IARI, New Delhi. Cultivation trials gave an average yield of 1.5 kg of tubers per plant after 18 months of crop duration. This strain has also done well in subtropical climate of Assam and at present this variety is being tested in multi-locational trials. *Pusa*-1 clones have been multiplied in sizable quantity and are ready for release and distribution (17ab).

Planting material and planting—The crop grows best from tuber pieces of 50-70 g weight; growth of the plants is poor when smaller pieces are used. For the sake of convenience, tubers can be divided into three portions: crowns, median portions, and tips (Fig. 2.5). The crowns sprout readily and normally sprouting is completed in about 30 days, while the median portions and tips may take about 100 days or so. Since direct planting in the field will mean less than optimum utilization of land for about 3 months and will necessitate incurring avoidable expenses and

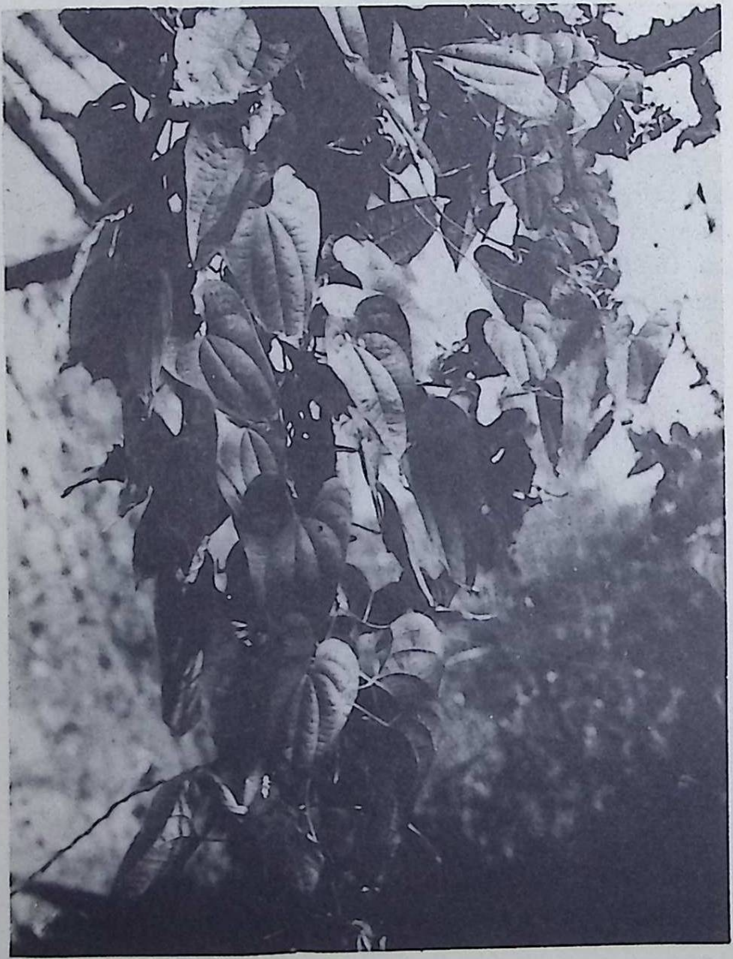


Dr R.K. Bammi, CIPLA, Bangalore

Fig. 2.5—*Dioscorea floribunda*: Portions of a tuber.

weeding, irrigation and general caring of the field, this method is not preferred for commercial cultivation of *D. floribunda* (3a).

For commercial planting, tuber pieces are stored for about a month before planting. To avoid tuber rot, the pieces may be dipped for 5 minutes in 0.3% solution of Benlate (3g/litre of water), followed by dusting of the cut ends with 0.3% Benlate in talcum powder before planting or storage. The Benlate-treated tuber pieces are kept in a raised bed in shade, covered with sand, and watered daily. After about 30 days the sand may be removed and the crown portions, which are now ready for sprouting in about a week's time, may be planted in the field. The left over median and tip portions are covered with sand and watered regularly. After about 60-75 days of the start of the storage, these pieces also are ready for planting in the field (3a).



Dr M.C. Tyagi, IARI, New Delhi

Fig. 2.6—*Dioscorea floribunda*: cultivated plant, *Pusa*-1.

It has been observed that under Bangalore conditions, the tuber pieces, including median portions and tips, sprout within a shorter period of 15-20 days if they are planted in late March or early April. This is so, perhaps, because of the higher temperature prevailing at that time. The tuber pieces, planted at other time of the year take longer to sprout. Crowns are preferred for commercial planting as they not only sprout earlier, but also contain less diosgenin than the median and tip portions, which may be retained for extraction if the crown portions suffice for propagation purposes (3a).

The land is ploughed thoroughly 3 or 4 times till a fine tilth is obtained and since requirement of organic manure is high, 20-25 tons of farm-yard manure/ha may be applied. A spacing of 60 cm between the furrows and 30-45 cm between the plants has been recommended (33a). The stored tubers are planted in furrows at a depth of 5-8 cm. The plant may be earthed up after about a month; converting the original ridges into furrows, and furrows into ridges, with plants in them. The support system tried successfully at Bangalore consists of stone pillars 1.5-2 m in height, spaced about 9 m apart in the field. Each plant is supported by gunny twines pegged to the ground and tied to the overhead wires (3a).

Fertilizers—It has been found that the strain *FB*(*C*-1) responds well to nitrogen. A complete fertilizer dose of 300-336 kg of nitrogen, 150-168 kg of P_2O_5 and 150-168 kg of K_2O can be applied per hectare. Phosphorus is applied as a basal dose at the time of planting. Nitrogen and potash should be applied in 4 split doses at intervals of 2 months, the first dose being given after two months of sprouting (3a, 33b).

Irrigation is given at a week's interval to begin with, followed by irrigation after about 10 days. No major pests and diseases have been observed. Infestation by mites was got rid of by the use of Akar or Kelthane (0.5%) (3a).

Duration of crop and harvesting—The size and weight of the tubers increase with age—on an average tubers from 1-year old plant weigh 1 kg and from a 2-year old plant, 3.5 kg. Diosgenin content of the tubers correspondingly increases from 2.5 to 4.0%. The cost of cultivation for second year's growth is also relatively less (3a). The tubers are 15-25 cm deep and can be harvested manually with pick-axes, or by deep ploughing.

Single-node leaf-cutting—Partial success by ground and air-layering was obtained in *D. floribunda* during the rainy season and the role of humidity in promoting rooting was recognized. The success with single-node cuttings, planted in a propagation chamber with intermittent mist, was consistent, but the percentage of rooting was low (20-30%). A quick dip in indole butyric acid, (5,000 ppm), followed by dusting with 0.2 per cent Benlate in talc gave the best results. Benlate acts as a disinfectant. The leaves from the median portion of the shoot responded better than basal and terminal leaves. The roots and shoots are formed in 30-40 days and the plants can be transferred to the field shortly afterwards (3a). CIPLA is using this method for clones where faster propagation is required for genetic and other specialized studies (3c). See also portion on Tissue Culture.

Crop from seeds—Commercial crop from seeds is recommended only when there is shortage of tuber material (3a), as the quality of the progeny from seed is uncertain. Fresh seeds give the highest germination. The optimum temperature for seed germination is 30°C. Planting medium for seed germination consists of equal amounts of soil, organic matter and sand. Seedlings may be staked immediately. They should be allowed to grow in the nursery for at least 6 months (Jan.-June) and then planted out in the field. The best time for transplanting is during the rainy season. A crop raised from seeds gives comparatively less yield and requires longer time (3 years) before it can be profitably harvested.

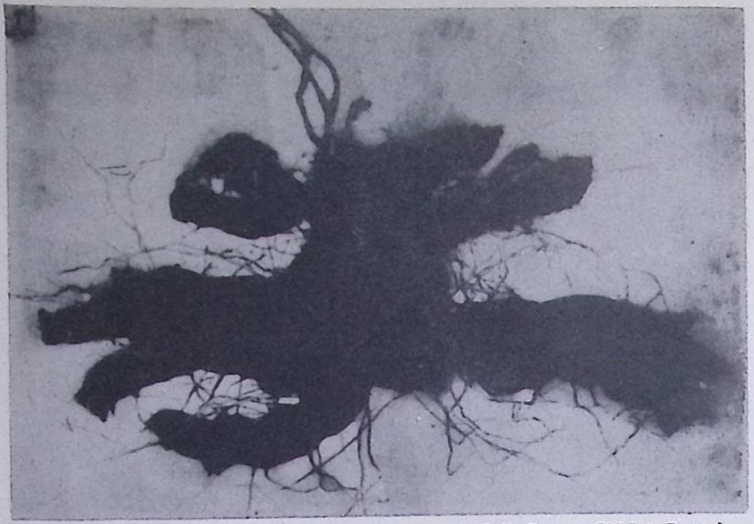
Marketing—Though some steroid drug companies have taken up the commercial cultivation of *D. floribunda* around Bangalore, they are also interested in having this crop grown on contractual basis. The minimum price guaranteed by the drug companies is Re 1.0 per kg of dry tubers with 1% of diosgenin. In other words if diosgenin content is 3%, the price allowed by the drug companies will be Rs 3 per kilo of dry tubers (3a).

Path analysis—The important components of the chemical yield in *D. floribunda* are not yet fully clear. Since the sapogenin is extracted from the underground tuber, the tuber yield, the dry weight of the tuber, and the percentage of diosgenin are important components. Knowledge of the interrelationships of these components and their direct and indirect effects on the yield of the sapogenin is, therefore, essential in a selection programme (3a).

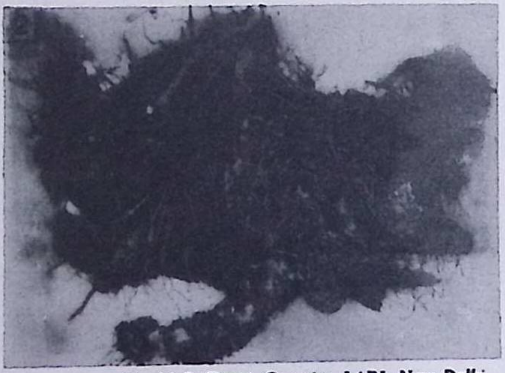
Yield performance and nature of character association in *FB* (*C*-1) strain propagated through tuber cuttings have been studied. The genotypes differ significantly in their mean values for diosgenin percentage, tuber yield, and yield of dry matter. Tuber yield is highly heritable, followed by the yield of sapogenin, dry matter, and diosgenin percentage. Phenotypic and genotypic correlation coefficients clearly established positive relationship between yield and its components (3a).

Partitioning of the correlation coefficients through path analysis indicated that tuber yield had pronounced indirect effect over the yield of the sapogenin through diosgenin percentage. The significant positive association of tuber yield with chemical yield is highly desirable for making a quick and easy selection, since the tuber yield has no unfavourable effect on diosgenin percentage. This relationship obviates the need for chemical analysis of each and every genotype in an extensive selection programme (3a).

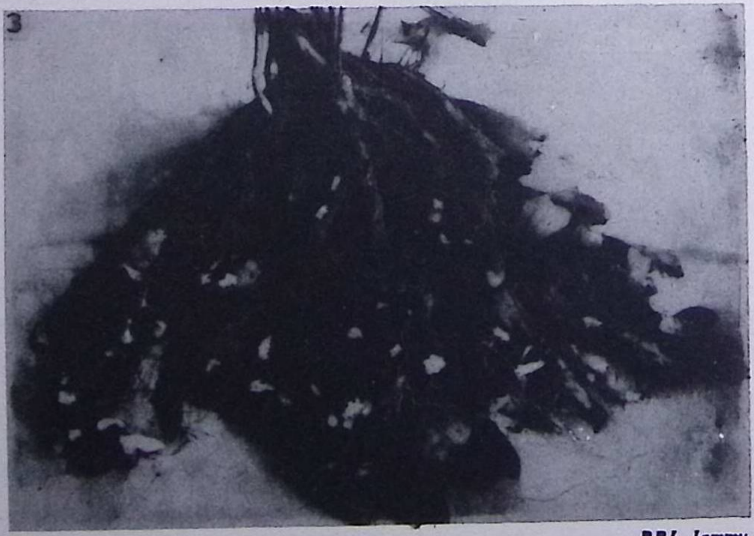
Pl II



Dr. R.K. Bammi, CIPLA, Bangalore



Dr. M.C. Tyagi, Genetics, IARI, New Delhi



RRL Jammu

DIOSCOREA TUBERS FROM CULTIVATED PLANTS

1, *D. floribunda*—FB (C-1), age 15 months, wt 2.5 kg; 2, *D. floribunda*—Pusa-1, age 20 months, wt 1.52 kg; 3, *D. composita*, age 36 months.

At the Symposium on "Production and Utilization of Vegetable Raw Materials for Steroid Hormones and Oral Contraceptives", held at the National Botanic Gardens, Lucknow in March, 1977, it was the concensus that cultivation of *D. floribunda* should be given the highest priority. IIHR, Bangalore; CIMPO, Lucknow; NBG, Lucknow; and IARI, New Delhi were charged with the responsibility of supplying the planting material to all prospective cultivators. It was also recommended that research programmes be undertaken for genetic upgrading of the planting material and improving cultivation practices to reduce the capital cost, and that plant protection measures be intensified.

D. composita Hemsl. (= *D. macrostachya* Benth.) (Fig. 2.7)

Though the valid botanical name is *D. macrostachya* Benth. (32), the more popular synonym *D. composita* has been used in this monograph.

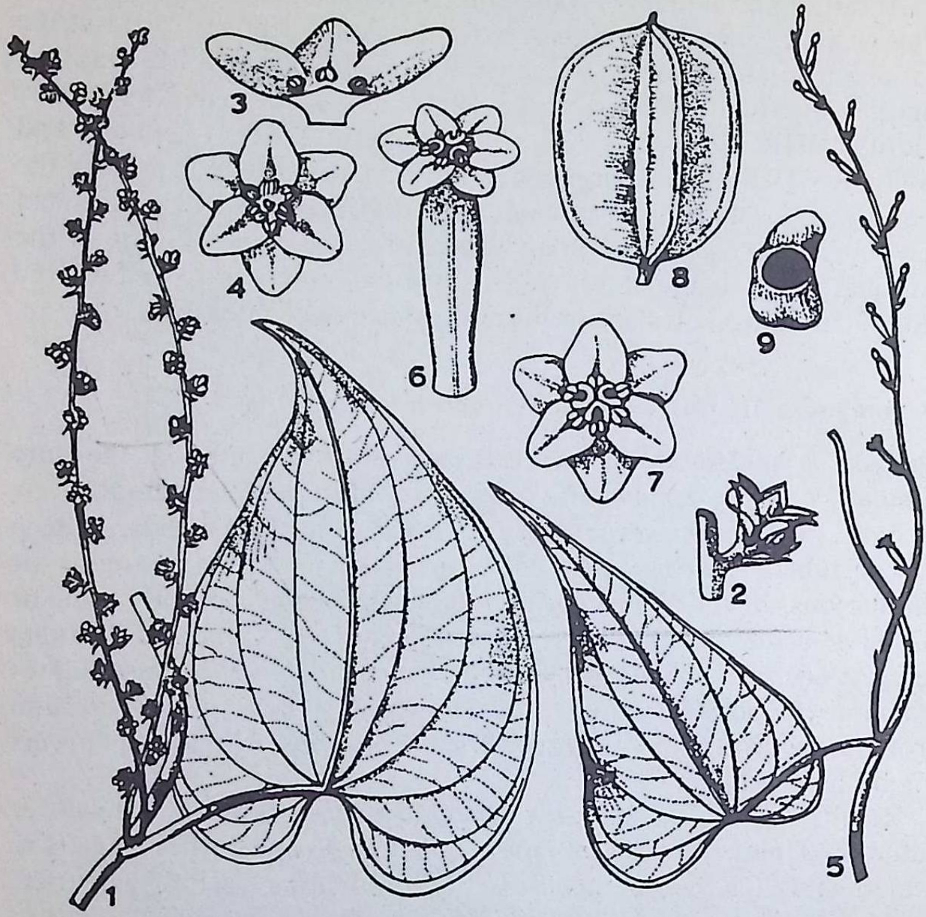
It is a nearly glabrous or glabrous, right-twining vine with large, deep rooted tubers. Leaves alternate, long petiolate, membranaceous or chartaceous, 20 cm × 18 cm, or usually much smaller, abruptly acute or cuspidate-acuminate, shallowly or deeply cordate, 7-9 nerved. Flowers dense, sessile, in staminate, simple or branched inflorescences, fasciculate-glomerate; fascicles short stipitate, 2-3 flowered; perianth rotate, 1.5-2 mm broad; male fascicles 15-30 cm long, fertile stamens, 6; in female flowers stigma bifid (32).

Results obtained so far indicate that the plant does well at Bangalore, and in the Union Territory of Goa (7), Jammu (9) and Jorhat (34a). It is also doing well in the Anaimalai Hills (27) and in the Darjeeling district.

Vines are robust and long; they often cross between supports and thus heavily shade the weeds to their exclusion. Tubers are white, large (*c.* 5 kg), long (*c.* 1.5 m) and deep (0.5 m) (Fig. 2.11). They are susceptible to frost. Diosgenin content is 4 to 6 per cent of the cultivated tubers in Puerto Rico (22). In India, the diosgenin content of the tubers from 2 to 3 years old crop is reported to vary from 2 to 4 per cent. Though tubers from 5-year old crop showed 7.5 per cent diosgenin content, cultivation was not found economical for the overall recovery of the sapogenin (27, 7).

Planting material—Tuber segments weighing approximately 50-60 g are initially planted in polythene bags filled with a mixture of soil and compost. Seedlings emerge after 35-40 days and are transplanted in circular pits of 80 cm diameter, at a depth of 40 cm. The pits are well prepared with a mixture of soil and compost—hay and cow-dung mixture in a ratio of 3:1, which has been allowed to decompose in pits for about 4 months (34a).

Since the crown portions from the tubers are easiest to propagate, they are recommended for commercial cultivation. Crown portion from all

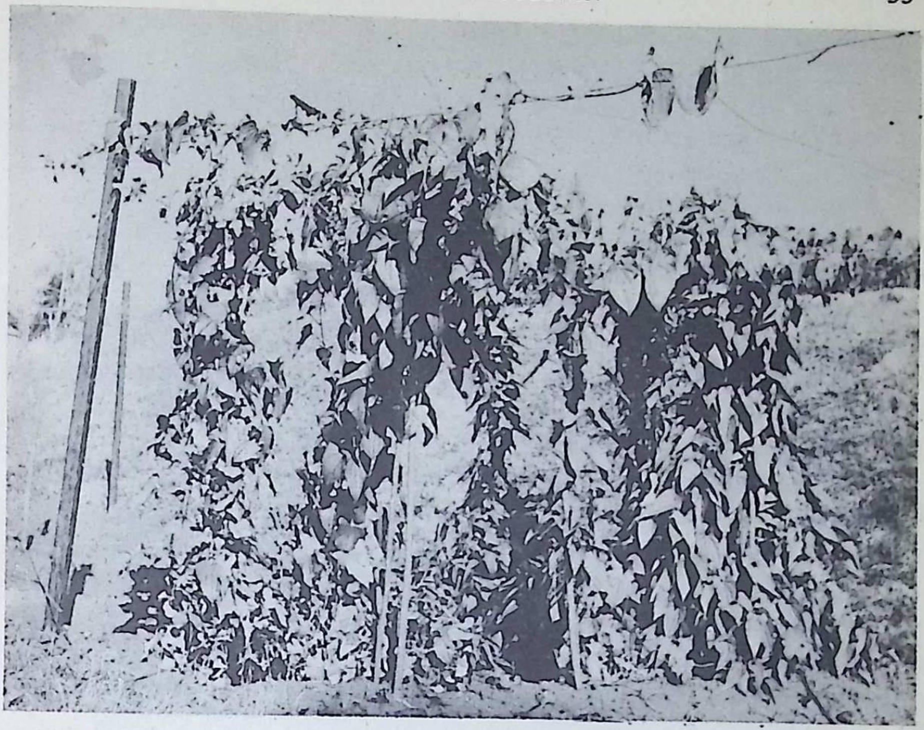


After Das Pflanzenreich, Heft 87, 1965, Fig. 34

Fig. 2.7—*Dioscorea composita* Hemsl. (= *D. macrostachya* Benth.): 1, A branch with male flowers; 2, A portion of the male inflorescence magnified; 3 & 4, Male flowers; 5, A branch with female flowers; 6 & 7, Female flowers; 8, Capsule; 9, Seed.

plants should, therefore, be kept for propagation purposes (3ab). However, according to Rabha *et al.* (34a), crown portion contains maximum diosgenin and not the tips contrary to the findings of Bammi & Randhawa (3a).

According to Prof. Chatterjee (36) when 100 to 200 g of *D. composita* tuber pieces are planted in a loose and nitrogen-rich soil in Darjeeling area, the growth of biomass is rapid. After one year the weight of tubers per plant is 8-10 kg; from a 3-year old crop, with the use of proper organic fertilizers as high as 30 to 40 kg of tubers with 3 to 4 per cent diosgenin (on dry wt) can be obtained per plant. Currently about 1.5 hectares of land is under *D. composita* cultivation at the foot-hills of Darjeeling district; plans to extend its cultivation in place of *D. prazeri* are afoot. Spacing required



RRL, Jammu

Fig. 2.8—*Dioscorea composita* under experimental cultivation.

for the enormous biomass of *D. composita* is much more than that for *D. floribunda*, with correspondingly less number of plants per hectare; as such the dry tuber yields from the two species may be almost the same. However, *D. composita* being a hardy plant does better than *D. floribunda* under severe conditions.

In experimental cultivation at RRL, Jorhat, mean weight of fresh tubers per plant propagated vegetatively from tuber pieces, was found to be 3.8, 9.8 and 14.3 kg in 1-year, 2-year and 3-year old crop respectively with an average moisture content of 71%; percentage of diosgenin for whole tubers was 2.28, 2.95 and 3.27 respectively (34a,b).

Crop from seeds—This method is practised by Cinchona Department, Ootacamund (27). Seeds are shown in alkathene bags filled with soil containing shola soil, sand, and local soil in the proportion of 2:1:1. The bags are stacked and sheltered and watering is done regularly. The seedlings are kept in bags for 5 months (Jan.-May) before planting in the field. *Rhizoctonia* sp., brings about Collar rot in the nurseries, which can be effectively controlled by Brassicol 0.5% suspension.

The area, after clearing the tree growth is converted into bench terraces (1 m width). Ridges (45 cm width) are built on these terraces (27).

The seedlings are planted during south-west monsoon with 1 m × 1 m spacings in furrows (10,000 plants per ha). The seedlings get developed into vines in about 30 days after planting and trellis construction is undertaken with bamboos and G.I. wire. About 3 rounds of weeding and 1 round of hilling up of earth are given in a year for optimum yield (27).

Fertilizers—The soil *p*H in Anaimalai hills is observed to be 5.5. A manure mixture of 50:14:112 NPK is applied in two split doses at the rate of 400 kg/ha to boost up the growth.

Harvesting and yield—The yield of diosgenin was only 2 per cent from the tuber after 40 months of the growth of the plant in Anaimalais (27).

Searle (India) have reported that the weight of tuber per plant was 8 kg in a 3½-year old crop, raised from the seed (35).

D. deltoidea

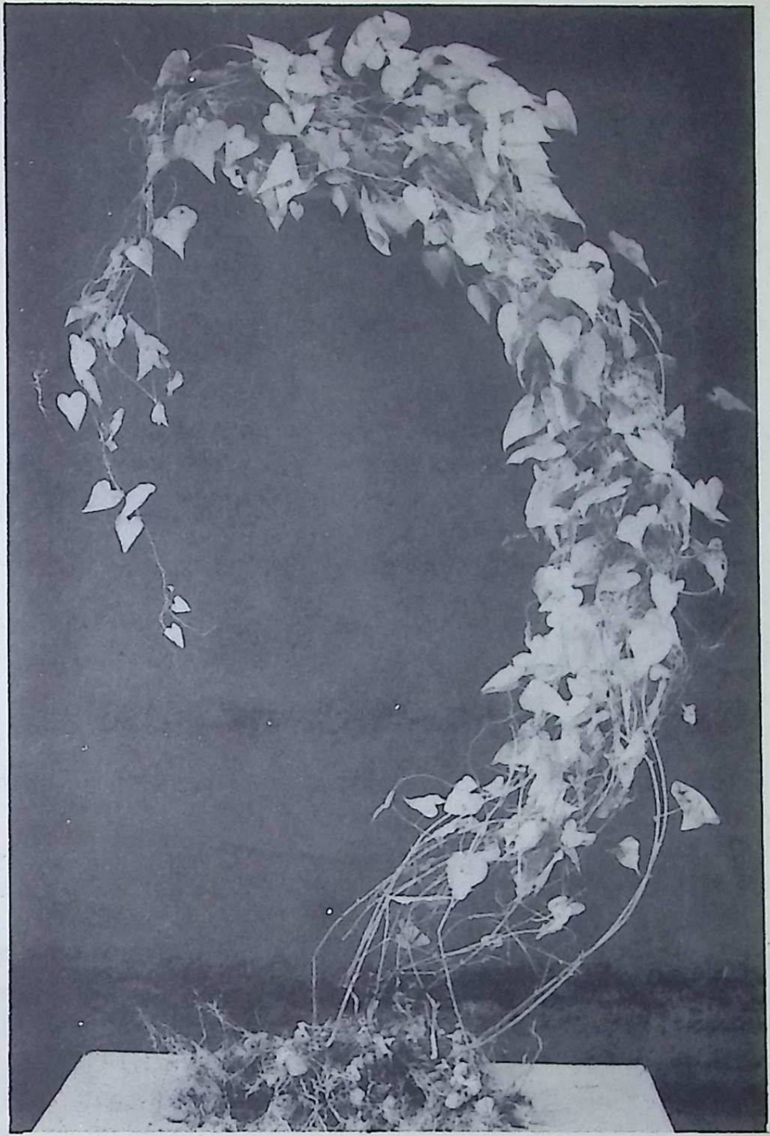
Research programmes are being carried out to bring under cultivation this obstinate but most important diosgenin-yielding plant. Results obtained so far at CIMPO Regional Centre, Manasbal (J & K) have shown that *D. deltoidea* can be successfully grown under field conditions (20b) and steps for its cultivation are being taken up at CIMPO's 120 hectare farm at Zainapore, Kashmir.

Crops grown from tubers obtained from wild plants came up reasonably well at Bangalore during the first year, but subsequent crops raised from Bangalore tubers gave discouraging results: the plants remained stunted and tuberization was poor. The results with *D. deltoidea* at Lucknow were equally disappointing (3b). Adaptation breakdowns, exhibited by this species, are not completely understood.

Tuber planting—About 2.4 tonnes of planting material is necessary for planting one hectare, when 50 g rhizome pieces are planted at spacing of 30 cm × 70 cm. The tubers should be dipped in 3 per cent solution of any fungicide, such as Cuman-1, for 5 to 8 minutes before planting (24).

For tuber-planting, the land is prepared by 3-cross-harrowings followed by tilling so as to obtain a fine tilth. Rhizomes of good quality are divided into 50 g pieces and planted in December. The field is kept free from weeds and is irrigated 6 times during the year from April to October. The plants are supplied with support of willow branches after the seedlings have reached a height of about 23-30 cm.

Fertilizers—The soil at Manasbal is medium loam, and poor in organic matter, phosphorus, nitrogen and potash. At the time of planting, one truck-load of 3 tonnes of well-rotten pyrethrum is added along with a basal dose of 80 kg/ha of phosphorus, 60 kg/ha of potash and 40 kg/ha of nitrogen. Three split doses of nitrogen at a rate of 20 kg/ha are applied as top dressings during the months of May, July and September during the



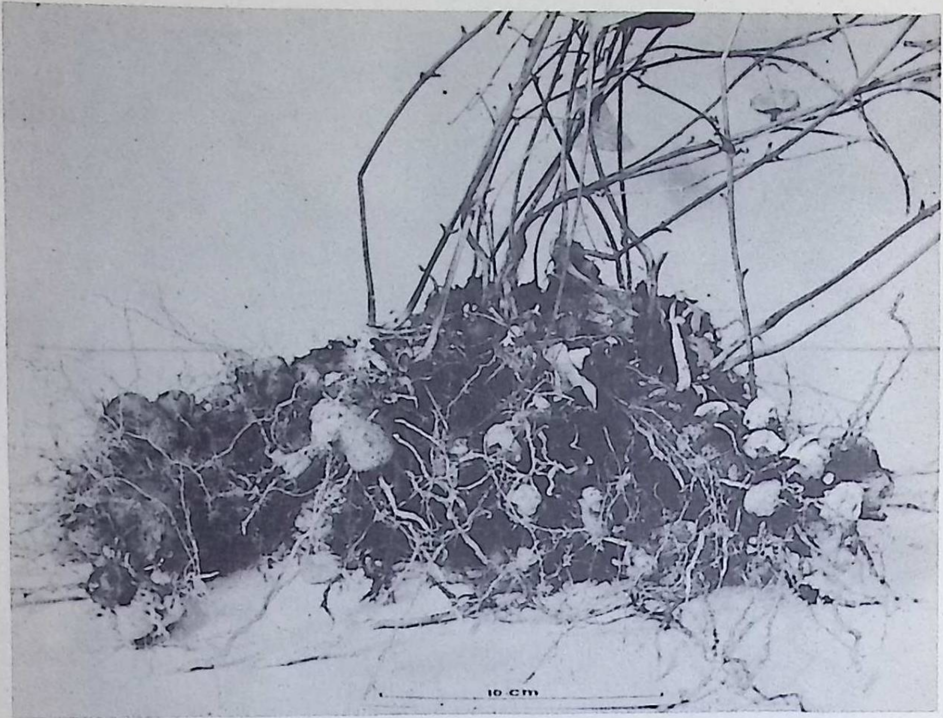
RRL, Jammu

Fig. 2.9—Cultivated *Dioscorea deltoidea* with tubers.

first year. The same fertilizer dose is repeated during the 2nd year. During the 3rd year, 60 kg nitrogen is applied in 2 split doses.

No pests and diseases have been observed.

Harvesting and yield—The crop is harvested in October, after a period of two years and ten months. Rhizomes are washed free of soil. A yield of 19.5 tonnes of fresh rhizomes or 7.5 tonnes of dry rhizomes (calculated on the basis of 60 per cent moisture content) per hectare can be obtained. Diosgenin content of harvested rhizomes ranges from 2.40 to 3.94 per cent



RRL, Jammu

Fig. 2.10—Tubers of *Dioscorea deltoidea* after 2 years' growth.

with an average of 3.28 per cent, which is almost the same as in the seed rhizomes.

Crop from seeds—Raised seed beds are made by mixing well rotten leaf manure or farm yard manure at the rate of about 10 baskets per 100 sq.m. The NPK fertilizers are mixed with organic manure. The size of the beds is 160 cm × 60 cm × 27 cm. About 1-1.5 kg of seeds per 100 sq. m are required. Germination starts after 20-25 days and is completed in a month's time. Regular watering is done by a can, or light irrigation is provided. The nursery beds are weeded manually when required. A dose of urea, about 250 g/100 sq. m, mixed with 1 kg of sand, is given to seedlings to avoid scorching. Seedlings with thumb-sized tubers are ready for planting out in the field (25). Observations at RRL, Jammu reveal that mature seeds, collected from forest area in the month of November, give up to 90 per cent germination after 3 months of storage; seedlings raised during March should be transplanted in July and August, or preferably in the subsequent March (12c).

D. prazeri

D. prazeri is reported to afford impure diosgenin (7,8) but according to Chatterjee (36), the plant growing in Darjeeling area (West Bengal) is a good source of diosgenin. Plantation of *D. prazeri* has been undertaken by the Medicinal Plants Directorate of West Bengal on an area of about 25 ha in Gairibas near Jaldhaka Hydel Project, in Darjeeling district. However, plans are afoot to replace it by *D. composita.* The output of green tubers (*c.* 75% moisture content) from a 3-year old crop of *D. prazeri* ranges between 12.5 to 17.5 tonnes per hectare. The diosgenin content is reported to be 2.5 to 3.5 per cent on dry wt basis (36).

D. prazeri is being tried by CIMPO, Lucknow on an experimental scale. Tubers with about 4.5 per cent diosgenin content have been introduced. The vines are reported to be healthy and growing very well. After about 8 months the yield was 95 g of fresh tubers per plant, with about 3 per cent diosgenin content on dry wt basis (20b). Karnick (37) obtained 700 g of fresh tubers (358 g dry weight) per plant, with 4.8 per cent diosgenin on dry basis after about 3 years of growth.

Economics of Cultivation

The operations involved for a cycle of 3 years for *D. deltoidea* cultivation, along with the actual yearly expenses are summarized in Table 2.6. It gives an idea of the cost of individual operations in the overall expenditure. The expenses in the first year of cultivation far exceed expenses in the subsequent year, and the expenditure is the least in the second year. Economics of cultivation of *D. floribunda* strains and *D. composita* are compared with *D. deltoidea* in Table 2.7.

TABLE 2.6—COST OF D. DELTOIDEA CULTIVATION*

	Cost (per hectare in Rs)			Total cost for 3 years
	1st year	2nd year	3rd year	
Land preparation	500	—	—	500
Planting	250	—	—	250
Irrigation	300	300	300	900
Weeding & hoeing	1,000	1,000	1,000	3,000
Fertilizers & their application	2,000	2,000	500	4,500
Support	1,000	—	—	1,000
Harvesting	—	—	1,000	1,000
Drying, cleaning and packing	—	—	700	700
Miscellaneous	—	—	150	150
Planting material	5,000	—	—	5,000
Interest on capital and land rent	—	—	1,000	1,000
Total cost	10,050	3,300	4,650	18,000

*Tajuddin *et al.*, *New Bot.*, 1976, 3, 44-47.

TABLE 2.7—ECONOMICS OF CULTIVATION OF DIOSCOREA SPP.

	D. floribunda[1,2,3]					
	FB (C-1) strain		Pusa-1	*D. composita*[4]		*D. deltoidea*[5]
	1-year	2-year	2-year	2-year	3-year	3-year
Dry tubers, yield tonnes/ha	7.5-10.5	16.5-18.0	11.25	10.7	15.4	7.8
Gross returns Rs/ha	22,500-31,500*	49,500-54,000*	33,750*	32,100*	46,200*	78,000**
Total expenses Rs	N.A.	N.A.	13,750	22,250	27,990	18,000
Net profit Rs/ha†	10,000-12,500	25,000-30,000	20,000	9,850	18,210	60,000

[1] Bammi & Randhawa, Dioscorea Improvement Project—Status Report, 1972, 12-13;
[2] Bammi *et al.*, Extension Ser. No.9, 1975 (IIHR, Bangalore);
[3] Tyagi *et al.*, *Intensive Agric.*, 1976, 15(8), 22-23;
[4] Information from Shri L.C. Rabha, RRL, Jorhat;
[5] Tajuddin *et al.*, *New Bot.*, 1976, 3, 44-47.

*Calculated at the rate of Rs 3 per kg of dry tubers, with 3% diosgenin.
**Calculated at the rate of Rs 10 per kg of dry tubers, the 1976 price of wild tubers.
†Interest on investment not taken into account.

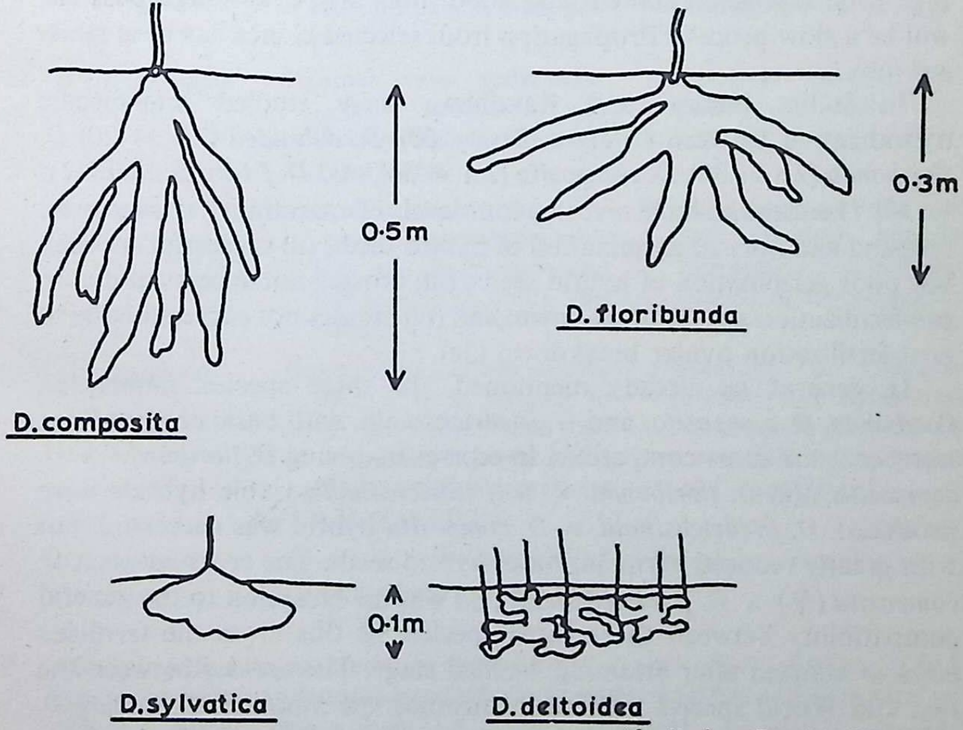
According to the figures in Table 2.7, profit from a 3-year crop of *D. deltoidea* is Rs 60,000 as against Rs 25,000 - 30,000 from a 2-year crop of *FB* (*C*-1) strain of *D. floribunda*, Rs 20,000 from a 2-year crop of *Pusa*-1 strain of *D. floribunda*, and Rs 9,850 from a 2-year and Rs 18,210 from a 3-year crop of *D. composita*. These returns do not take into account the interest on the capital investment. Moreover, the data pertain to mid- or early-seventies, and since then there has been appreciable fluctuation in prices of the tubers. The figures of expenditure and returns on dioscorea cultivation, therefore, give only rough estimates.

Many difficulties are encountered during dioscorea cultivation, the most important being the exacting climatic and soil requirements of the plants, and the long time of about 3 years between sowing and harvesting of the crop. The other problems include heavy expenditure on the installation of supports for the dioscorea vines, and on weeding, fertilizers, and harvesting. No definite varieties have been developed, and the environmental factors play a major role in determining the yield. Therefore, the strains selected for superior performance in a particular region do not necessarily give better yields than the non-selected strains (3,22).

GENETICS, BREEDING AND PLANT IMPROVEMENT

Cytological studies reveal that *Dioscorea* species from the New World have a basic chromosome number of 9, whereas the Old World species have 10. Consequently, strong isolating barriers have been reported between the Old World (*D. deltoidea*, 2n = 20) and New World species (*D. floribunda*, *D. composita*, *D. friedrichsthalii*, *D. spiculiflora*, 2n = 36), and crosses between them have not generally been successful. In general, the four American species are cross compatible (3a, 22).

Many genes are involved with the yield of dioscorea tubers. All the species that are amendable to cultivation are dioecious and are tetraploids with 36 chromosomes. Fixing these genes into superior varieties by conventional breeding techniques would be difficult. Since environment plays an important role, breeding and selecting superior varieties would be a long and tedious process, requiring many generations to complete the process. Four *Dioscorea* species, viz. *D. composita*, *D. floribunda*, *D. friedrichsthalii* and *D. spiculiflora* have been extensively studied and found to vary considerably in their breeding behaviour. As such, strains selected for superior performance in a particular region do not necessarily give better results than the non-selected strains (3a, 22). Moreover, species itself is a mixture of poor to excellent plants, and such differences within



After Hardman, Trop. Sci., 1969, **11**, 204

Fig. 2.11—Different shapes of the tubers of wild *Dioscorea* spp., diagrammatic.

the species are not visible. The desirable and undesirable characters of some important *Dioscorea* species are summarized in Table 2.8.

Hybridization—Considerable research work on breeding of *Dioscorea* species for high diosgenin content and good tuber shape and yield has been carried out in the U.K., India and Puerto Rico (6b). The major objective of hybridization studies is to combine in the hybrids the desirable attributes of the different species. Since dioscoreas can be propagated vegetatively, fixation of any hybrid vigour, which may manifest itself, is possible though it is difficult to achieve (3a).

In the U.K., for example, the cross *D. deltoidea* × *D. sylvatica* was successful. The former has a higher diosgenin content, but has branched tubers, not well-shaped for planting and harvesting, whereas the latter has conical compact tubers (Fig. 2.11). The F_1 and F_2 generations had a diosgenin content similar to that of *D. deltoidea* and the tubers showed compactness similar to those of *D. sylvatica* (6b).

A good deal of breeding research work on *Dioscorea* species has been carried out by Martin and coworkers in Puerto Rico. One of the most promising crosses obtained as a result of their work was *D. composita* × *D. spiculiflora*, which showed hybrid vigour and disease resistance. Martin has outlined a possible breeding programme, suggesting that selection for high total sapogenin content and good tuber shape, although possible, will be a slow process. Propagation from selected clones has been stressed (6b).

In India, Bammi and Randhawa have studied interspecific hybridization between *D. alata* ($2n = 60$), *D. deltoidea* ($2n = 20$) *D. floribunda* ($2n = 36$), *D. composita* ($2n = 36$), and *D. friedrichsthalii* ($2n = 36$). Their studies have revealed four levels of crossability: (i) successful crossing and normal germination of hybrid seeds; (ii) successful crossing but poor germination of hybrid seeds; (iii) crosses not successful due to pre-fertilization ovular breakdown; and (iv) crosses not successful due to post fertilization ovular breakdown (3a).

In general, as already mentioned, the three species, namely *D. floribunda*, *D. composita*, and *D. friedrichsthalii*, with basic chromosome number 9, are cross compatible. In crosses involving *D. floribunda* × *D. composita*, and *D. floribunda* × *D. friedrichsthalii*, viable hybrids were produced. *D. friedrichsthalii* × *D. composita* hybrid was successful, but with greatly reduced germination of hybrid seeds. The cross between *D. composita* (♀) × *D. friedrichsthalii* (♂) was an exception to the general compatibility between these three species. In this cross the fertilized embryo aborted after attaining 4-celled stage. The crosses between the two Old World species with basic chromosome number 10, namely *D. alata* and *D. deltoidea*, and with the above named three American species were not successful (3a).

TABLE 2.8—DESIRABLE AND UNDESIRABLE GROWTH CHARACTERISTICS OF SOME IMPORTANT DIOSCOREA SPP.

Species	Desirable	Undesirable
D. alata[1]	Very high-yielding—up to 15 kg of tubers/plant/year; aerial tubers for propagation present.	Contains no diosgenin; tubers soft and therefore rot easily.
D. composita[1]	Vigorous vines, moderate tuber yield and diosgenin content; can also be propagated from single-node leaf-cuttings.	Tubers rot easily; grow up to 45 cm deep in the soil; tuber dormancy present; branched tubers.
D. deltoidea[1]	Tubers contain high amount of diosgenin in pure form; easy to propagate from tuber pieces; tubers shallow and horizontal—easy for harvesting; hardy tubers.	Weak growing, low tuber yields; branched tubers.
D. floribunda[1]	Vigorous vines; high tuber yields—1-2 kg/plant/year; moderately high in diosgenin content; can be propagated from single-node leaf-cuttings and tuber pieces.	Tuber pieces rot easily; they take up to 3 months to sprout; harvest somewhat difficult as tubers grow up to 25 cm in the soil; branched tubers.
D. friedrichsthalii[1]	Vigorous vines under Bangalore conditions moderately high in diosgenin, white tubers.	Relatively low in tuber yields (under Bangalore conditions).
D. prazeri[2]	Easy to propagate from rhizome pieces; rhizomes shallow and horizontal—easy for harvesting; hardy rhizomes.	Weak growing, low tuber yield (0.5 kg); highly branched rhizomes; impure diosgenin; content of sapogenins moderate.
D. spiculiflora[3,4]	Easy to propagate from juvenile rosettes; quite resistant to diseases (under Puerto Rico conditions), Tubers white, sapogenin percentage very high (8 to 14%)	Propagation from tubers not suitable; yield of tuber low; very impure diosgenin (accompanied by gentrogenin and correllogenin).
D. sylvatica[5]	Conical-shaped, compact tubers without branching; easy for harvesting.	Diosgenin content low.

[1] Bammi & Randhawa, Dioscorea Improvement Project—Status Report, 1975, 80; [2] Information through personal communication; [3] Martin & Gaskin, *U.S. Dep. Agric. prod. Res. Rep.*, No. 103, 1968, 5; [4] Wall *et al.*, *J. Am. Pharm. Assoc., Sci. Ed.*, 1957, **46**, 155-59; [5] Blunden *et al.*, *Trop. Sci.*, 1975, **17**, 139-54.

(i) *D. floribunda* × *D. composita*—These hybrids yielded on an average 0.50 kg, to a maximum of 1.20 kg of tubers in 2 years from seeds. Some of the tubers had completely white flesh, while other exhibited intermediate colours. Heterosis was observed for leaf size, leaf number, stem diameter, side branching, flower size, seed size, and tuber yield in one year. A comparative account of characteristics of *D. floribunda* and *D. composita* and their hybrid is given in Table 2.9.

(ii) *D. composita* × *D. floribunda*—The mean tuber yield was 0.48 kg and the maximum 1.15 kg. There were no reciprocal differences.

(iii) *D. floribunda* × *D. friedrichsthalii*—The mean tuber yield of this cross was low (0.17 kg); the colour of the tuber flesh was intermediate between the parents, at the periphery white gradually becoming yellow.

(iv) *D. friedrichsthalii* × *D. floribunda*—The mean tuber yield was 0.50 kg. There were no other reciprocal differences.

Prefertilization breakdown was observed in all crosses involving *D. alata* (the cultivated species yielding edible tubers, with no diosgenin) and *D. deltoidea*, between themselves and also with the Central American

TABLE 2.9—COMPARISON OF CHARACTERISTICS OF D. FLORIBUNDA AND D. COMPOSITA AND THEIR HYBRID*

Character	*D. floribunda*	*D. composita*	F
Leaf size	Small medium	Medium large	Medium large
Leaf variegation	Nil-highly variegated	Nil	Nil-highly variegated
Leaf number	Low-high	Medium	High
Leafiness	Low-high	Low medium	High
Sprouting	Rapid	Slow	Rapid
Diameter of stem	Small	Large	Very large
Side branching	Sparse	Sparse	Profuse
Vigour rating	Highly vigorous	Vigorous after one season of growth	Highly vigorous
Shape of the tuber	No prominent neck	Prominent neck	Intermediate
Colour of tuber flesh	Yellow	White	White-yellow
Flowering season	Sept.-Dec.	June-Jan.	July-Dec.
Degree of flowering	Medium	Profuse	Profuse
Flower size	Medium	Medium	Large
Fruit size	Medium	Large	Large
Seed size	Medium	Normal	Large
Dry season dormancy	Marked	Less marked	Less marked
General drought tolerance	Average	High	High
Tuber yield in one year	High	Low	High

*Bammi & Randhawa, Dioscorea Improvement Project—Status Report, 1975, 17.

species. The reciprocal crosses of *D. deltoidea* with the Central American species gave different results. It was observed that when the diploid *D. deltoidea* was used as the female parent and the male parent was polyploid, e.g. *D. deltoidea* (♀) × *D. floribunda* (♂), a prefertilization breakdown took place as the ♂ pollen tubes fail to reach and fertilize the ovules. In reciprocal crosses, e.g. *D. floribunda* (♀) × *D. deltoidea* (♂) fertilization did take place, but the embryo aborted at 4-celled or 8-celled stage.

In situations where there is post fertilization breakdown, it is felt that embryo could be dissected out and at least some hybrids could be produced by *in vitro* culture of the embryo. In cases, where pollen tubes fail to reach and fertilize the ovules, in order to obtain hybrids, pollination with irradiated pollen or intra-ovarian fertilization holds promise (3a).

A number of hybrids between *D. floribunda* and *D. composita* have been evolved by CIPLA, and of these about 12 are under multiplication and further trials. Some of the hybrids, unlike *D. floribunda*, do not go into senescence in January and February, but like the other parent, *D. composita*, stay evergreen under Bangalore conditions. The average diosgenin content of *D. floribunda* clones which CIPLA is commercially growing now is 3%, but some of the hybrids have as high as 5% diosgenin (3c).

Mutation Breeding—Mutation breeding in *D. floribunda* through gamma irradiation and two chemical mutagens, viz. N-nitroso methyl urea (NMU) and diethyl sulphate (DES) has been studied by Murthy & Rao (38a). Chemical mutagens were more effective in altering the mean values, DES being more effective than NMU. NMU was found to stimulate germination and seedling vigour.

The observations by these workers made on genetic variance and mean values for germination and survival percentage, dimensions of the first seedling leaf, plant height (3 months) and seedling vigour in the M_1 generation suggest the possibility of altering genetic structure of this important medicinal crop through mutation breeding. The variation in advanced generations should be more, as has been the case with other crops like rice, wheat and *Toria* (*Brassica campestris* Linn. var. *toria* Duthie & Fuller).

Meiotic Studies—In the studies aimed at the improvement of dioscoreas, it was observed that the chromosomes in *D. floribunda*, *D. composita* and *D. friedrichsthalii* were very small ranging from 0.3 μ to 1.5 μ at M_1. Meiosis was regular, with regular formation of 18 bivalents. Since the basic chromosome number of these species is 9 and meiosis is regular, they may probably represent allotetraploids ($2n = 36$) containing two genomes, the homologies of which are not known.

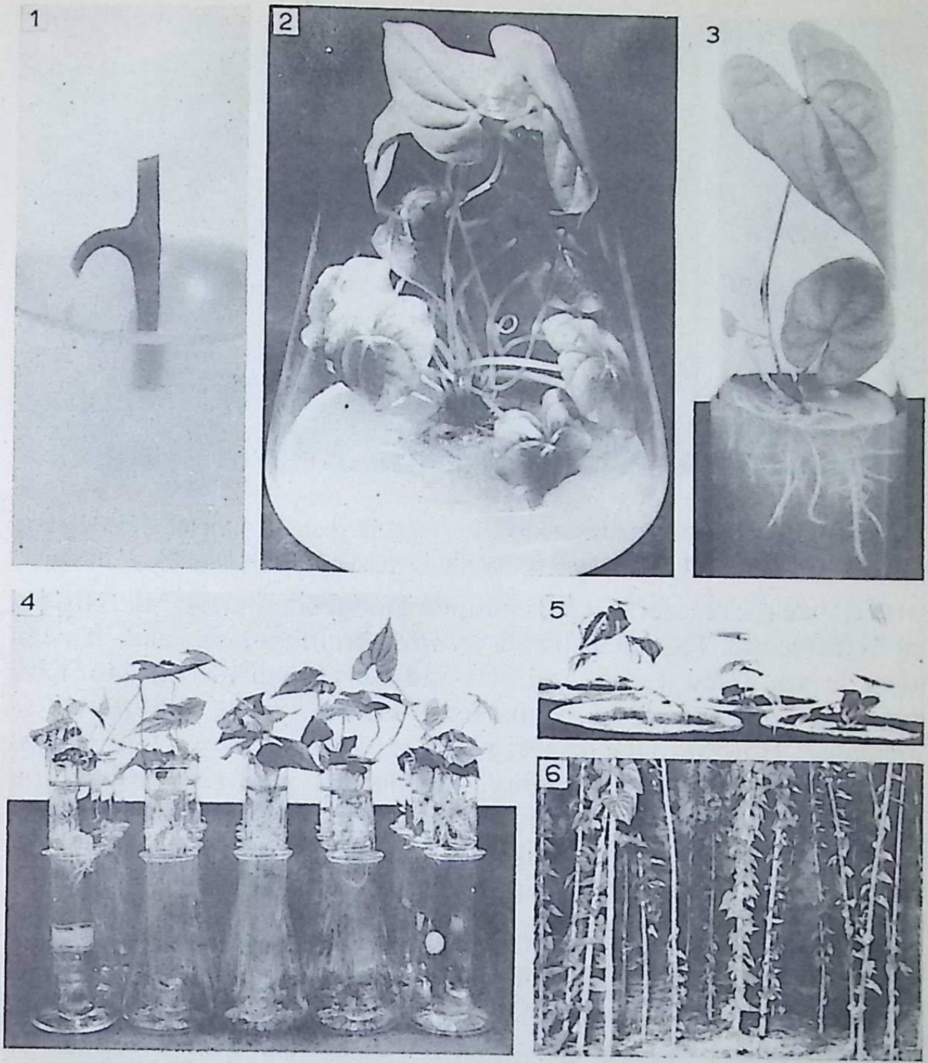
The hybrids had fairly regular meiosis except for the presence of a few univalents and higher associations involving 3 and 4 chromosomes. It could not be made out whether these chromosome associations represent multivalents of these formed due to reciprocal translocations. Meiotic behaviour of the species alone, however, does not rule out completely the former possibility, since such a diploid behaviour of the species could result from a genetic control of chromosome pairing or a gradual diploidization of homo or homoeologous genomes. When such genomes are brought together in a new combination, it is quite possible for the original homologies to get expressed due to an altered genetic background. The male and female fertilities of the hybrids lend less support to the existence of reciprocal structural changes (3a, 38b).

TISSUE CULTURE

In India, the tissue and organ culture work on plants containing diosgenin has been pursued along two lines, viz. (i) propagation of plants involving morphogenetic studies, and (ii) biosynthesis of diosgenin in callus cultures.

The main limitation in achieving the large scale cultivation of dioscoreas has been the slow-propagating nature of these plants. Hardly 10 plants can be obtained from the tuber of one plant after a year. Whereas some success in regenerating the stem cuttings of *D. floribunda* by usual horticultural methods has been reported by Bammi & Randhawa (3a), no headway has been recorded for propagation of *D. deltoidea.*

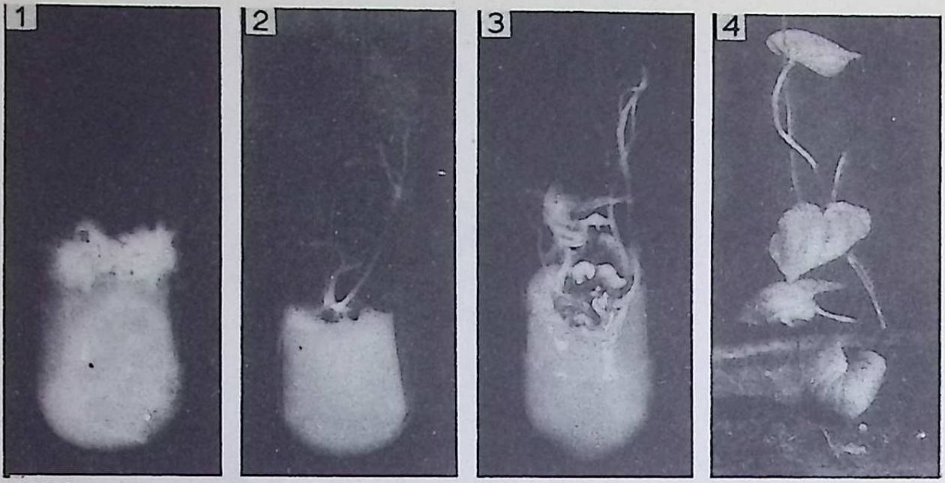
Chaturvedi (39a), for the first time, demonstrated the possibility of rapid clonal propagation of *D. floribunda* through tissue culture by achieving 100 per cent rooting of single-node leaf-cuttings. In this process, which is of special relevance for initial multiplication of elite clones, the plants established in aseptic cultures (in flasks) through shoot apices, or single-node stem segments, taken from the field-grown vines, comprised the experimental material for further propagation. About 40 single-node leaf-cuttings were obtained from each such aseptically grown plant and when cultured in a rooting medium, they all rooted within a period of 20-30 days, resulting in as many plants. Furthermore, the same number of cuttings could repeatedly be obtained after every second month, from each flask by subculturing the remaining rooted base of plants. In this way, an enormous true-to-type planting material could be obtained in a short time. It took 40-50 days to obtain 5 to 6-leaved plantlets in potted soil from cuttings taken from an aseptically grown plant. The *in vitro* raised plants produced normal tubers after transplantation in soil under field conditions. Similar results have been obtained by *in vitro* culturing of only the excised leaves, i.e., without exillant buds (Chaturvedi and Sinha, unpublished data).



NBRI*, *Lucknow

Fig. 2.12—Clonal propagation of *Dioscorea floribunda* through *in vitro* culture: 1, A single-node stem-segment in aseptic culture; 2,A plant in aseptic culture; 3, Rooting of a single-node leaf cutting taken from an aseptically grown plant; 4, Acclimatization of tissue-plants in an inorganic salt solution; 5, Acclimatized plants in potted soil; 6, One-year-old *in vitro* raised plants in the field.

Recently, a confirmatory report has been published by Sita *et al.* (40) emphasizing the value of tissue culture for rapid propagation of *D. floribunda*. Again, Chaturvedi *et al.* (39b) for the first time demonstrated clonal propagation of *D. deltoidea* by *in vitro* culture of shoot apices and single-node leaf cuttings. Regeneration of plants through shoot apex culture is of great significance, as *Dioscoreas* are reported to be affected by a number of virus diseases. Chaturvedi and Sinha (unpublished data) have



NCL, Pune

Fig. 2.13—Tissue culture of *Dioscorea deltoidea*: 1, Callus culture; 2, Shoot initiation from macerated tuber; 3, Multiple shoot formation from callus; 4, Tissue-plant in pot soil.

also shown the regeneration of multiple plants from somatic callus tissues of *D. floribunda*. The callus plants showed variation in size and shape of their leaves. Grewal and Atal (41) and Mascarenhas *et al.* (42) have reported regeneration of plants from somatic callus cultures of *D. deltoidea*. However, the exact mode of differentiation of organized structures in callus tissue, leading to the formation of plantlets was not elucidated in these publications. Chaturvedi and Sinha (unpublished data) have shown that actually the cells of the differentiating callus cultures of *D. deltoidea* follow the embryogenetic pathway.

Biosynthesis of diosgenin in callus cultures—Isolation of crystalline diosgenin (0.7%) from callus of mature tuber tissue of *D. deltoidea* by Chaturvedi and Srivastava (43) disproved the belief that the tuber functions only as a storage organ for diosgenin. Recent studies by Chaturvedi and Chowdhury (unpublished data) have shown that callus from the tissue of very young tubers of *D. deltoidea* synthesized higher amount of diosgenin (1.6%).

In steroidal production and bio-transformation, suspension cultures have been found to be more useful than static callus cultures, the greater intimacy with the medium gives a greater sensitivity to medium composition, the presence of cells in suspension allows easier separation of cells from medium, with the medium also being used as a potential source of required constituents. The cells may also be maintained in the post lag phase of culture by continuous monitoring of media composition and cell number. This gives a higher proportion of actively growing cells with a more efficient utilization of precursors, such as cholesterol and subsequent conversions into steroidal compounds (44).

Tissue culture work on *Dioscorea* is also being carried out in the U.S.A., particularly by Staba and his coworkers. Kaul, Stohs and Staba (45) achieved as high as 2.6 per cent of diosgenin in *D. deltoidea* suspension cultures by the addition of cholesterol and yeast extract of RT - 0.1 (RT—Revised Tobacco Medium of Murashige and Skoog). A high concentration (0.5%) of yeast extract resulted in greater induction of diosgenin production per gram dry weight, but this effect was nullified by a decrease in cell growth. Recently Marshall and Staba (46) analysed *D. deltoidea* tissue cultures grown in the presence of 2,4-D, indole-3-butyric acid, isopentyladenine, benzyladenine, and gibberelic acid (GA) singly and in combination. They observed that the medium with 2,4-D most consistently favoured diosgenin production, although the value was not as high as 2.6 per cent, obtained in suspension cultures by the addition of cholesterol and yeast extract combination. GA and high benzyladenine concentrations were toxic.

An attempt to develop an industrial medium for tissue cultures of *D. deltoidea* has been made (47).

So far the tissue culture work has been of academic importance only. It is now being geared to solving urgent practical problems of propagation of elite selections on a commercial basis.

OTHER GENERA

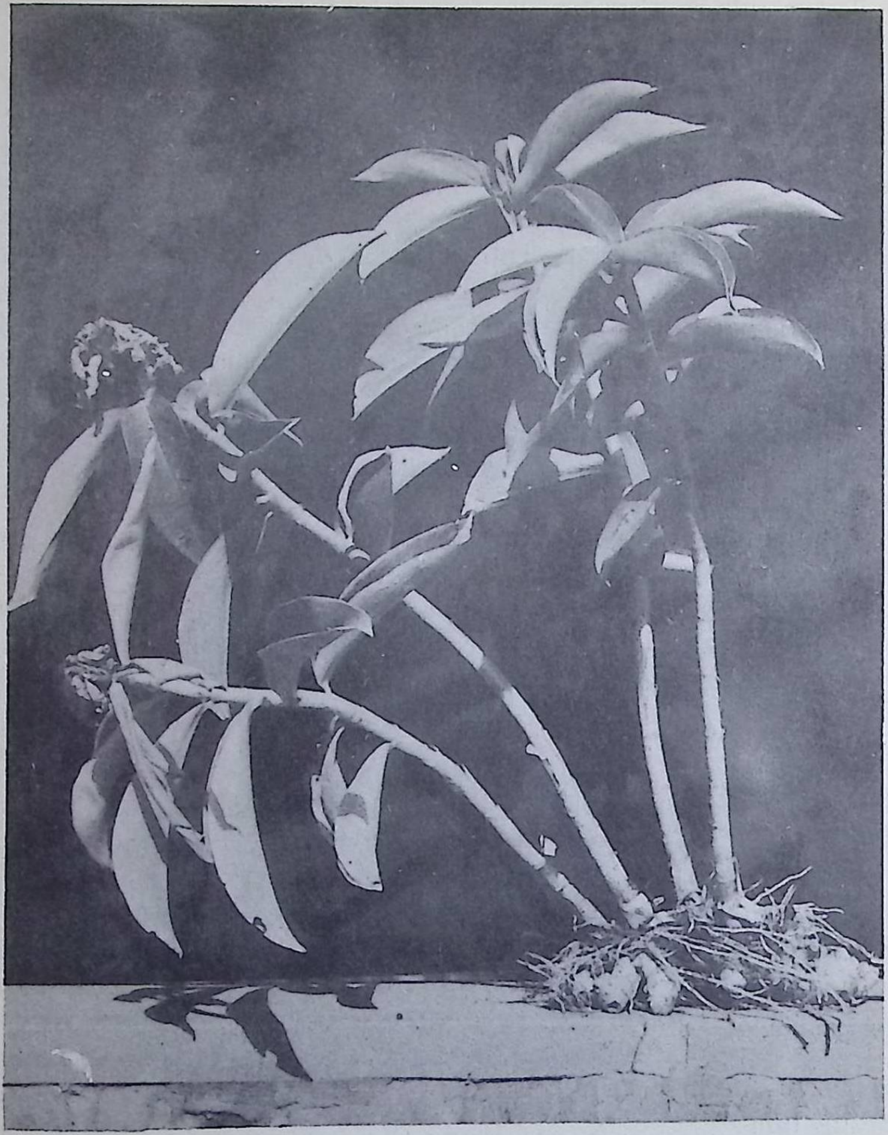
The cultivation problems of dioscoreas, dealt with earlier, indicate that other plants with lower yields of the genin, but a shorter growing cycle, might also prove economical sources of diosgenin (48,49). In India, the species most likely to be grown for this purpose appear to be *Costus speciosus* (Koenig) Smith (50) and *Kallstroemia pubescens* (G.Don) Dandy (51). The well known spice fenugreek, *Trigonella foenum-graecum* Linn., which is being studied in Kenya and Morocco (49) as a source of diosgenin is also receiving attention in India (7). The wild growing *Balanites aegyptiaca* (Linn.) Delile is another promising material under investigation.

Among other potential sources elsewhere, mention may be made of *B. wilsoniana* Dave & Sprague, *B. orbicularis* Sprague, *B. pedicellaris* Mildbr. & Schlecht. (all fruits); *Paris polyphylla* Smith (rhizomes); and *Agave ellemeetiana* A. Jacobson (6b, 5c).

Costus speciosus Sim.

It is a common herb with a tuberous rhizome, distributed throughout India up to an altitude of about 1200 m.

Names in Indian languages—Sanskrit: *Kemuka*; Hindi & Bengali: *Keu*; Marathi: *Penva*, *Pushkarmula*; Telugu & Kannada: *Chengalvakoshtu* (5b).

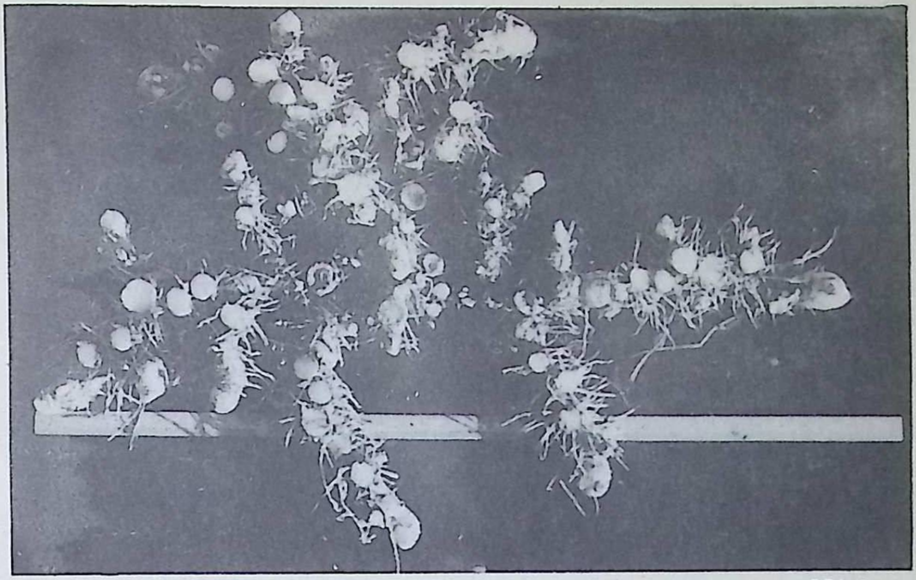


RRL, Jammu

Fig. 2.14—*Costus speciosus*: the whole plant.

The plant is a perennial erect, stout herb, attaining a height of 1.2-1.7 m. Rhizome tuberous horizontally branching, up to 5 cm thick when fresh. Leaves oblong or lanceolate, about 30 cm long, silky beneath, spirally arranged on thick cane-like stems. Flowers in dense terminal heads, large white, with yellowish centres, usually with wide red bracts (5b,50).

Sarin *et al.* (50) of RRL, Jammu have studied distribution, habitat, and diosgenin content of the species. In its natural habitat, the plant grows in



RRL, Jammu

Fig. 2.15—*Costus speciosus*: Tubers harvested from 18-month-old plant.

moist, partially shady localities of semi-deciduous forests. The herb is more common in the sub-Himalayan tract from Kangra district of Himachal Pradesh eastwards to Arunachal Pradesh; in Khasi, Jaintia and Garo hills of Meghalaya; Vindhya and Satpura hills in Central India; upper catchments of river Godavari; and in the Western Ghats in Maharashtra, Karnataka and Kerala (5b, 50).

Diosgenin content — Chemical analysis of a large number of rhizome samples, obtained from various parts of the country, has indicated a wide range in the diosgenin content from 0.32 to 3.37 per cent.

Evaluation from the point of view of diosgenin content showed that the samples obtained from the sub-Himalayan belt of Jammu, Kangra (Himachal Pradesh), Dehra Dun (Uttar Pradesh) and Hazaribagh (Bihar) were better in quality than those obtained from the Western Ghats and Orissa. Diosgenin of 95 per cent purity has been reported.

The highest yield of diosgenin is obtained in the month of July when the plant is in active stage of growth and flowering has just started. There is a sharp decline in September when the plant is in full bloom and fruit formation starts in the lowest parts of the floral heads. There is further decline during December, after which the genin content again gradually increases up to April or May.

Cultivation—Cultivation trials have been carried out by Sarin *et al.* (50). Rhizome cuttings, each with at least two viable buds, serve as good planting material, the average weight of such rhizome pieces varying from

35 to 60 g. Rhizomes are selected from high diosgenin-yielding plants. About 1500 kg. of fresh rhizomes/ha are required. Propagation through seeds is uneconomical.

For planting, the land is thoroughly prepared by at least four ploughings, and organic manure is applied to the soil at the rate of 15 tonnes per hectare. As the herb is shallow-rooted, the manure should not leach into deeper layers. A basal dose of 40 kg/ha of super phosphate at the time of planting is recommended.

A spacing of 75 cm × 75 cm is given for a crop to be harvested after 15 months. It can be reduced where the crop is to be harvested after a period of 6 to 9 months.

The rhizome pieces are placed horizontally about 10 cm deep in the ridges, the buds facing upwards. The optimum sprouting (above 95%) occurs during the months of April and May; planting during March and June shows a high rate of desiccation of young sprouts, requiring copious irrigation.

The crop planted between April and May requires at least three or four irrigations a month. In the post-monsoon period the frequency of irrigation is reduced to two per month. One weeding in the first month of sprouting followed by one or two earthings, keep the crop fairly free from weeds. During the second year, only one or two weedings in April or May are required.

Harvesting and Yield — Rhizomes contain the highest quantity of diosgenin when the plants are in flowering stage. They are dug out with spades and left overnight in the open to loosen the earth sticking to the rhizomes.

The yields from 6 months, 9 months, and 20 months crops are 413 g, 665 g, and 1,773 g of fresh tubers per plant respectively, with 80-85 per cent moisture content. Large scale trials have given a yield of 4.0 tonnes of dry rhizomes/ha on harvesting after 15 months. The diosgenin content was 1.7%, giving 68 kg of diosgenin/ha.

Prospects—The advantages of *C. speciosus* over dioscoreas are: (i) Short-growing cycle of 6-15 months as compared with about 2-3 years in dioscorea cultivation; (ii) ease of cultivation; (iii) simple staking; (iv) enormous biomass (rhizomes) obtained in short duration (48); and (v) the same machinery and chemicals as are used for the extraction of diosgenin from *Dioscorea* spp., can be used for *C. speciosus.*

Prospects for commercial cultivation of *C. speciosus* as an alternative source of diosgenin in India are reported to be good (12c). However, for this purpose it is necessary to breed strains giving high yields of rhizomes and diosgenin. It may be pointed out that diosgenin yield of 68 kg/ha from *C. speciosus* is far short of 180 kg/ha (average) from 1-year old harvest of *D. floribunda.*

According to Chatterjee(36), cultivation of *Costus* species has shown promise at the foot-hills of the Eastern Himalayas in the district of Darjeeling; the diosgenin content is however, low (1.5% only).

Kallstroemia pubescens (G. Don) Dandy

It is a tropical American plant which was introduced into West Bengal around 1955 and has become naturalized as a weed in the districts of Burdwan, 24 Parganas, hooghly, Midnapur and Nadia. Workable yields of diosgenin have been obtained from *K. pubescens* by Chakravarti *et al.* (51).

The plant has a reduced stem and spreads out radially like a trailer all along the ground. Leaves compound with three pairs of small leaflets; flowers small, 6-7 mm in diameter, white to light yellow in colour, blooming in the morning and withering by midday; fruits small, hard and thorny (51).

It is difficult to locate the plant in the wild as it does not rise above ground level appreciably. In the morning, however, the plant can easily be seen because of the profusion of tiny flowers.

Sapogenins of *K. pubescens* consist almost entirely of diosgenin, which is present in free as well as in the glycosidal form. The yield of diosgenin from the whole herb collected from different areas in India varied from 0.96 to 1.71 per cent, and that of the leaves from 1.78 to 1.92 per cent on dry wt basis. The plants from Burdwan gave the maximum yield.

A number of advantages of this plant as a source of diosgenin are observed: (i) very pure diosgenin; (ii) easy cultivation; (iii) a short cycle of about six months as compared to *Dioscorea* or even *Costus* sp.; (iv) easy collection of the weed by simple scraping or combing as compared to digging out of tuber from ground; and (v) easy grinding of the plant material; and (vi) fermentation-cum-acid hydrolysis method suitable.

Trigonella foenum-graecum Linn. Fenugreek

Fenugreek is widely cultivated throughout India as a spice. It is being investigated as a possible source of diosgenin though the seeds of Indian origin are low in the genin content.

Names in Indian languages—Sanskrit: *Methica*; Hindi, Bengali, Marathi, Gujarati:*Methi*; Telugu: *Merilikoora* (herb), *Metnthulu* (seeds); Kannada: *Mentesoppu*, *Menk-palle*, *Mente*; Tamil: *Vendayam*; Malayalam: *Uluva*, *Venthiam*.

An aromatic annual 30-60 cm tall, found wild in Kashmir, Punjab, and the Upper Gangetic Plains and widely cultivated in India for fodder and as a spice. Leaves 3-foliolate; leaflets 2.0-2.5 cm long, oblanceolate-oblong, obscurely dentate. Flowers white or yellowish white, 1 or 2, axillary. Pods 3-15 cm long, 10-20 seeded; seeds greenish brown, 2.5-5.0 × 2.5-3.5 mm, oblong, with a deep groove across one corner, giving the seeds a hooked appearance (5d).

The seeds are obtained in 3 to 5 months after sowing, depending on the climate. In some areas, two crops are grown annually. When grown entirely for seeds, an average crop of common *Methi* yields 1.5-1.9 tonnes of seed/ha (5d). The plant is fairly drought-resistant and is not subject to any serious fungal disease and pests (6b).

According to a recent investigation (52), seeds from Kangra (Himachal pradesh) contained 1.02 per cent total sapogenins with diosgenin and yamogenin (C-25 epimers) (53) in the ratio of 57:43; both the genins are acceptable to steroid drug manufacturers in a mixture or separately. Work carried out at the University of Bath, England, sponsored by Tropical Products Institute (TPI), London, has shown that the seeds contain 0.7 to 1.7 per cent of a mixture of diosgenin and yamogenin. A collaborative study between TPI and Kenya is in progress to establish whether fenugreek seed could be used for steroid production (4).

Cultivars, hybrids and mutants of *T. foenum-graecum* with increased resistance to pests and a higher yield of seed with high diosgenin content have been developed. Seed yields of 3.7 tonnes per hectare have been consistently achieved at Bath, England (6b). This quantity of fenugreek with 1% sapogenins will yield around 35 kg/ha diosgenin/yamogenin per crop or 105 kg/ha with 3 crops a year. The yield of diosgenin from fenugreek with 3 crops a year is much more than that obtained from *Costus speciosus* during the same period.

Diosgenin content as high as 1.82 per cent has been obtained by Khanna and Jain (54) in *T. foenum-graecum* callus cultures. By incorporating cholesterol in the medium, Khanna and coworkers (55) could enhance the diosgenin content up to 3.5 per cent.

Fenugreek seed can be a source not only of diosgenin, but also of protein (26%), a fixed oil (7%) with properties similar to linseed oil and a mucilage (gum). In fact, the herb could be grown primarily for its seeds for use as an animal feedstuff, recovering diosgenin and other materials as by-products. The integrated processing of the seeds will involve removal of the husk for its mucilage; extraction of the drying oil from dehusked seeds; and alcoholic extraction of the genin from the defatted meal. The residue would be a high-protein animal feedstuff. The hay would also be available for animals (49, 6b).

Prospects—Fenugreek scores over other plants in many ways: (i) it is a well-established cultivated crop; (ii) not susceptible to pests and diseases of serious nature; (iii) a short duration crop, produces seeds after a short period of about 3 months; (iv) unlike other plants, no plant part or plant product will go waste; (v) fenugreek is one of the very few sources where the genin would be available as a by-product and consequently may prove to be a cheap source; and (vi) it increases the soil fertility (being a legume) — this advantage is not shared by any of the known plant sources of diosgenin.

Judging from the above advantages, development of high-yielding strains both in terms of seeds and in genin-content within the country or their introduction from abroad for large scale cultivation warrants strong consideration. Some work on these lines has already been initiated by CIPLA (8).

The disadvantages in respect of fenugreek as a source of diosgenin are the dependence on alcoholic extraction, complete removal of fat before assaying of the genins, and purification step may be necessary but these are more than offset by several other advantages.

Balanites aegyptiaca (Linn.) Delile (Figs. 2.16 & 2.17)
syn *B. roxburghiana* Planch Desert Date

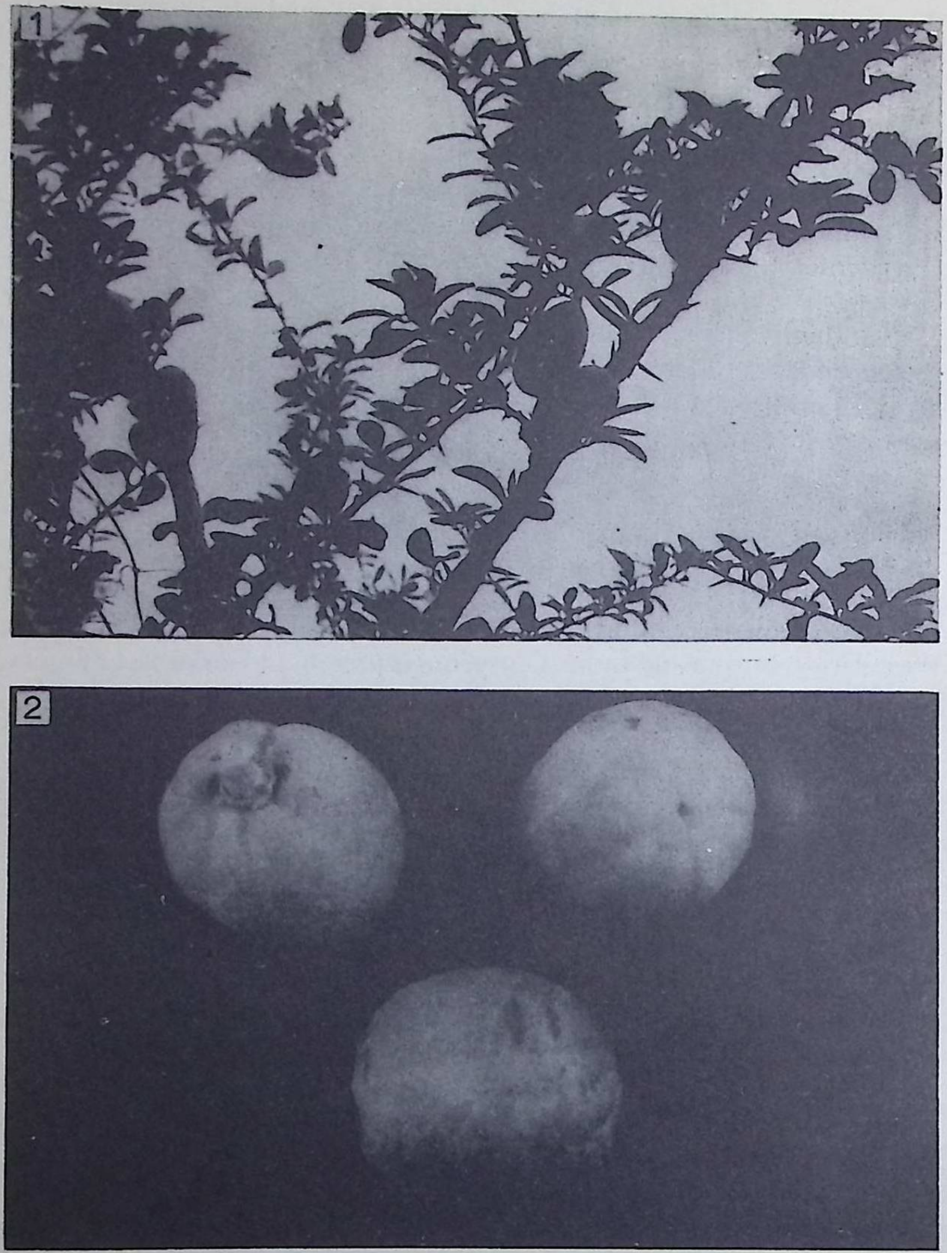
It is an evergreen tree occurring in a wild state in Arabia, Egypt, East and West Africa, Burma and India. Diosgenin is present in workable amounts in the pericarp of the fruits and in the seeds; it is generally accompanied by yamogenin.

Names in Indian languages—Sanskrit: *Ingudi*; Hindi: *Hingan*, *Hingot*; Marathi: *Hinganabet*; Gujarati: *Begorea*; Telugu: *Gari*; Tamil: *Nanjunda*.

A spiny tree about 6 m in height, with glabrous or puberulous branches ending in very strong, sharp, ascending spines. Leaves bifoliate, leaflets elliptical or obovate, coriaceous; cymes 4-10 flowered; fruits edible, one-seeded oily drupes, up to 5 cm in length, yellow when ripe during the winter months. The tree is characteristic of the drier parts of Gujarat, Rajasthan, Haryana, Uttar Pradesh, Bihar, Maharashtra and Deccan. It thrives on black cotton soil but not in rocky areas (5c).

All parts of the plant contain diosgenin/yamogenin mixture (57b, 58ab, 59). According to latest Indian work (57a) the fruit wall of the material obtained from Jodhpur contains 1.5 per cent diosgenin on fresh weight basis by Brain *et al*.s' method; this is a commercially acceptable quantity for fresh material. The availability of fruits and possibility of growing high-yielding plants are being assessed. According to information from Central Arid Zone Research Institute, Jodhpur, this tree growing wild in un-inhabitated and protected areas bears very many fruits.

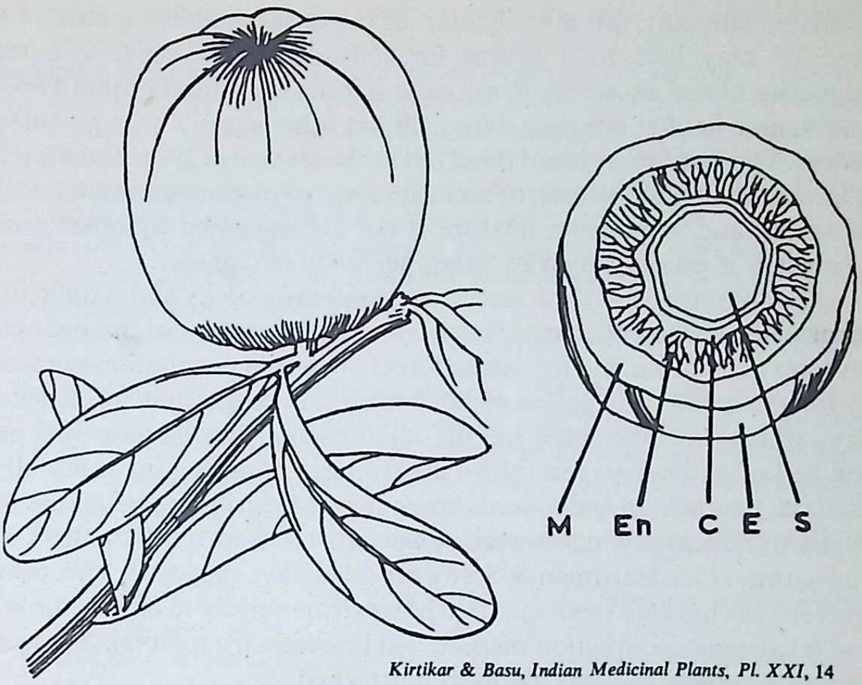
The fruit wall of unripe but full grown fruit of Egyptian origin, which is smaller than Indian fruit, has been found to contain 4.08 per cent diosgenin/yamogenin (in 44:56 ratio), on dry wt basis (58a).



Dr H.S. Mann, CAZRI, Jodhpur

Fig. 2.16—*Balanites aegyptiaca*: 1, Fruiting branch; 2, Dried fruits.

The seeds (oil 36-50%, protein up to 55%) may serve as a source of diosgenin after extraction of oil (60,56,61). Seeds from Jodhpur contained 0.6 per cent diosgenin on fresh weight basis (57b). A sample from Lucknow showed about 5.4 per cent of amorphous saponin (alcoholic extraction prior to fat extraction was followed) containing 3 genins of which



Kirtikar & Basu, Indian Medicinal Plants, Pl. XXI, 14

Fig. 2.17—*Balanites aegyptiaca*: Fruit and its transverse section—E, Epicarp; M, Mesocarp; En, Endocarp; C, Cavity; S, Seed

diosgenin was the major and the only crystalline genin. Saponin(s) can be recovered from the defatted meal by alcoholic extraction method and hydrolysed to yield corresponding genin(s). The residual meal can be used as an animal feed or as a fertilizer, and the oil for the manufacture of soap (58a, 56, 5c).

It may be mentioned here that IR spectrophotometric determination of total sapogenin as well as proportion of yamogenin, gives highly inflated figures when crude extracts are used particularly in the presence of fixed oil component. The extent of exaggeration can be seen from the following study. For instance, a sample of *B. aegyptiaca* seed previously defatted so as to remove 44% of the seed mass but still containing some fat gave diosgenin + yamogenin value as 2% with the proportion of 74:26. The extract when purified by column chromatography gave diosgenin + yamogenin value as 1% with the proportion of 92:8 (62). The purification of the crude extract is, therefore, absolutely necessary when fat is present along with sapogenin.

The roots are reported to contain up to 2.2 per cent diosgenin (57a) but obviously they cannot be used as a source of diosgenin.

Advantages over other plants— (i) Diosgenin/yamogenin mixture is available from the fruit wall and the seeds, as one of the by-products and the plant may therefore prove a cheap source of genin; (ii) the fruits are available annually; (iii) investigation of the unsaponifiable matter of the seed oil may lead to a source for steroid drug precursors, namely stigmasterol and sitosterol. *B. aegyptiaca* fruits may thus become 'two-in-one' source to offer not only diosgenin but other steroid drug precursors as well. This possibility is not ruled out as the seed oil of *B. orbicularis* (63a) is known to contain sitosterol and stigmasterol in good amount; and (iv) diosgenin and yamogenin mixture is not accompanied by other genins whereas it is accompanied by other genins in fenugreek.

Disadvantages — (i) The mesocarp is extremly sticky and is difficult to separate from the endocarp. The method of separation of the mesocarp involves incubation and for extraction of diosgenin, incubation-cum-acid hydrolysis method; (ii) period of incubation may range from 3-4 days to 21 days, which is cumbersome; (iii) the edible carbohydrate-containing pulp will be lost as food; (iv) endocarp is extremely hard making it literally a hard nut to crack. In India, seeds are removed by drilling small holes into the hard endocarps which are then made into crackers by filling them with gun-powder (5c). Hardman & Sofowora (58a) have suggested a procedure whereby the nuts are sawn into two halves transversely to release the seed; and (v) alcoholic extraction method will be necessary to obtain diosgenin from the kernels to keep the meal untouched.

REFERENCES

1. International Trade Centre UNCTAD/GATT Markets for Selected Medicinal Plants and their Derivatives. Geneva, 1974. Chapter IV—Dioscorea and Diosgenin, 68-69.
2. Coursey, D.G. Yams (Longmans, Green & Co. Ltd., London), 1967, 2, 35-42.

3a. Bammi, R.K. and Randhawa, G.S. Dioscorea Improvement Project—Status Report (Indian Institute of Horticultural Research, Bangalore), 1975; (3b) Status Report, 1972, 18, 61-62, 76; (3c) Dr.R.K. Bammi, Director, CIPLA Agricultural Research Division, Bangalore.

4. Gerasimenko, I.I. & Tropova, E.F. Introduction of *Dioscorea deltoidea* as a more prospective diosgenin source in the USSR. *Rastit. Resur.*, 1971, **7**, 192-200.

5a. The Wealth of India—Raw Materials, A Dictionary of Indian Raw Materials (Council of Scientific & Industrial Research, New Delhi), Vol. III, 1952, 67-76; (5b) Vol. II, 1950, 360; (5c) Vol. I, 1948, 143-44; (5d) Vol. X (Publications & Information Directorate, CSIR, New Delhi), 1976, 299-306.

6a. Hardman, R. Pharmaceutical products from plant steroids. *Trop. Sci.*, 1969, **11**, 196-228; (6b) Blunden, G., Culling, C. & Jewers, K. Steroidal sapogenins: a review of actual and potential plant sources. Ibid., 1975, **17**, 139-54.

7. Information through personal communication or discussion.
8. Information from Dr.Y.K. Hamied, Managing Director, CIPLA, Bombay.
9. Gupta, S., Sobti, S.N. & Atal, C.K. Introduction of *Dioscorea composita* Hemsl., a potential source of diosgenin in Jammu. I. Proc. Second Workshop of AICIP on Medicinal and Aromatic Plants, held at Anand, on November 1-4, 1976, 173-75.
10. Prain, D. & Burkill, I.H. An account of the Genus *Dioscorea* in the east. Part I. The species which twine to the left. *Ann. R. Bot. Gard.. Calcutta*, 1936. **14**(Part I), 25-30.

11. Abrol, B.K. & Kapoor, L.D. Distribution of kins (*Dioscorea deltoidea* Wall.) in north-western Himalayan region. *Bull. Reg. Res. Lab. Jammu*, 1962-63, **1**, 30-31.

12a. Sarin, Y.K. & Atal, C.K. *Dioscorea deltoidea*: present position and future prospects as raw material for diosgenin (Paper presented at NBG, Lucknow—Symposium on Production and utilization of vegetable raw materials for steroid hormones and oral contraceptives, held on March 26-28, 1977) (Abstract); (12b) Sarin, Y.K. Raw Materials for Indian steroid industry. *Indian Drugs*, 1974, **11**(10), 15-17; (12c) Information from Dr. Y.K. Sarin, Scientist, RRL, Jammu-Tawi.

13. Information from Dr.S.K. Jain, Director, Botanical Survey of India, Calcutta.

14. Information from Dr.A.S. Rao, Joint Director, Botanical Survey of India, Calcutta.

15a. Information from the Chief Conservator of Forests, J & K Govt., Srinagar; (15b) Himachal Pradesh, Simla; (15c) Nagaland, Kohima; (15d) The Director, Forests & Soil Conservation, Mizoram, Aizwal.

16. Information from Shri O.P. Virmani, Scientist, CIMPO, Lucknow.

17a. Tyagi, M.C., Sharma, B., Singh, M.P. & Jain, H.K. Clonal selection in *Dioscorea floribunda*. Proc. Second Workshop of AICIP on Medicinal and Aromatic Plants, held at Anand, on November 1-4, 1976, 214-18; (17b) Tyagi, M.C., Sharma, B. & Singh, M.P. Yam can check baby-boom. *Intensive Agric.*, 1976, **15**(8), 22-23.

18a. *Report Central Indian Medicinal Plants Organisation, Lucknow*, 1965-73, 6; (18b) *CIMPO Annu. Rep.*, 1973-74 & 74-75, 15, 2.

19. Chakravarti, R.N., Chakravarti, D. & Barua, A.K. Utilisation of Indian dioscorea plants for preparation of cortisone. *Indian Med. Gaz.*, 1953, **88**, 1-18.

20. Chopra, R.N., Badhwar, R.L. & Ghosh, S. Poisonous Plants of India (Indian Council of Agricultural Research, New Delhi), 2nd revised & enlarged ed., Vol. II, 1965, 866-67.

21. Information from Dr.G. Thyagarajan, Director, RRL, Jorhat.

22. Martin, F.W. & Gaskins, M.H. Cultivation of the sapogenin-bearing *Dioscorea* species. *U.S. Dep. Agric., Prod. Res. Rep.*, No. 103, 1968.

23. Wilson, A.P. & Mapother, H.R. A suggested method for *Dioscorea deltoidea* in temperate regions for the production of diosgenin. *Rep. Long Ashton Res. Stat.*, 1969, 203-07; 1970, 4 (through Abstract Bibliography of *Dioscorea* 1931-1972, prepared by Bammi *et al.*, December, 1972).

24. Tajuddin, Ram, P. & Husain, A. Cultivation of *Dioscorea deltoidea* as a raw material for diosgenin in Kashmir. *New Bot.*, 1976, **3**, 44-47.

25. Information from Shri B.C. Gulati, Scientist-in-Charge, Drug Research Laboratory (CIMPO), Jammu-Tawi.

26. Information from Dr. Rajendra Gupta, Project Coordinator, All India Coordinated Improvement Project on Medicinal and Aromatic Plants, IARI, New Delhi.

27. Information from Shri K. Doraswamy, Director, Cinchona Department, Ootacamund, Tamil Nadu.

28. Ruppel, E.G., Delpin, H. & Martin, F.W. Preliminary studies on a virus disease of a sapogenin-producing *Dioscorea* species in Puerto Rico. *J. Agric. Univ. Puerto Rico*, 1966, **50**, 151-57.

29. Alconero, R. Control of *Dioscorea* greenbanding virus with Demeton foliar sprays. *Plant Dis. Rep.*, 1969, **53**, 450-52.

30a. Schieber, E. & Dorothea, L.K. Root knot nematodes on *Dioscorea* in Guatemala. *Plant Dis. Rep.*, 1961, **45**, 981; (30b) Schieber, E. Parasitic nematodes on *Dioscorea* in Guatemala. Ibid., 1961, **45**, 425.

31a. Bruhn, C. & Koch, W. Control of root-knot nematodes in *Dioscorea* tubers. *Phytopathology*, 1963, **53**, 24 (abstr.); (31b) Koch, W. *Helminthosporium* leaf spot of *Dioscorea* in Guatemala. Ibid., 1964, **54**, 365-66.

32. Knuth, R. Dioscoreaceae, *in* Das Pflanzenreich, edited by Engler, A. [im Verlag von H.R. Engelmann (J. Cramer), Weinheim/Bergstrasse], No. 87, 1965, 180-82; 164-66.

33a. Rao, G.G., Randhawa, G.S., Bammi, R.K., Selvaraj, Y. & Subash, C.M. Effect of varying spacing on tuber yield and diosgenin content of two-year old crop of *Dioscorea floribunda. Indian J. Agron.*, 1976, **21**, 71-72; (33b) Rao, G.G., Selvaraj, Y., Bammi, R.K. & Randhawa, G.S. Effect of different levels of nitrogen and potash on yield and diosgenin content of *D. floribunda. Indian J. Hort.*, 1975, **32**, 168-70.

34a. Rabha, L.C., Chaudhuri, S.B. & Bordoli, D.N. Growth & diosgenin contents of *Dioscorea composita* Hemsl. under Jorhat conditions. *Indian Drugs*, 1976, **13**(11), 10-12; (34b) Information from Shri L.C. Rabha, Scientist, RRL, Jorhat.

35. Information from the Managing Director, Searle (India) Ltd., Bombay.

36. Information from Prof. S.K. Chatterjee, Director, Cinchona & Other Medicinal Plants, Govt. of West Bengal and Director, West Bengal Phytochemical and Pharmaceutical Development Corporation, Calcutta.

37. Karnick, C.R. Seasonal periodicity of sapogenin production in *Dioscorea deltoidea* Wall. & *D. prazeri* Prain & Burkill. *Planta Med.*, 1968, **16**, 269-72.

38a. Murthy, U.R. & Rao, V.R. Mutation breeding in medicinal yam *Dioscorea floribunda* Mart. and Gal. *Indian J. Hort.*, 1976, **33**, 274-77; (38b) Rao, V.R. & Murthy, U.R. Meiotic studies in species and hybrids in *Dioscorea. Curr. Sci.*, 1975, **44**, 24-25.

39a. Chaturvedi, H.C. Propagation of *Dioscorea floribunda* from *in vitro* culture of single-node stem segments. *Curr. Sci.*, 1975, **44**, 839-41; (39b) Chaturvedi, H.C., Sinha, M. & Sharma, A.K. Clonal propagation of *Dioscorea deltoidea* Wall. through *in vitro* culture of shoot apices & single-node leaf cuttings, *in* Cultivation & Utilization of Medicinal and Aromatic Plants, edited by Atal, C.K. & Kapur, B.M. (Regional Research Laboratory, Jammu-Tawi), 1977, 500-05.

40. Sita, G.L., Bammi, R.K. & Randhawa, G.S. Clonal propagation of *Dioscorea floribunda* by tissue culture. *J. Hort. Sci.*, 1976, **51**, 551-54.

41. Grewal, S. & Atal, C.K. Plantlet formation in callus cultures of *Dioscorea deltoidea* Wall. *Indian J. Exp. Biol.*, 1976, **14**, 352-53.

42. Mascarenhas, A.P., Hendre, R.R., Nadgir, A.L., Ghugale, D.D., Godbole, D.A., Prabhu, R.A. & Jagannathan, V. Development of plantlets from cultured tissues of *Dioscorea deltoidea* Wall. *Indian J. Exp. Biol.*, 1976, **14**, 604-06.

43. Chaturvedi, H.C. & Srivastava, S.N. Diosgenin biosynthesis by tuber-callus tissue cultures of *Dioscorea deltoidea. Lloydia*, 1976, **39**, 82-83.

44. Turner, T.D. Plant tissue culture as a source of phytopharmaceuticals. *Indian Drugs*, 1977, **14**(6), 112-24.

45. Kaul, B., Stohs, S.J. & Staba, E.J. Influence of various factors on diosgenin production by *Dioscorea deltoidea* callus and suspension cultures. *Lloydia*, 1969, **32**, 347-59.

46. Marshall, J.G. & Staba, E.J. Hormonal effects on diosgenin biosynthesis and growth in *Dioscorea deltoidea* tissue cultures. *Phytochemistry*, 1976, **15**, 53-55.

47. Mehta, A.R., Staba, E.J. & Grewal, S. Development of an industrial medium and selection of strains for high diosgenin production by *Dioscorea deltoidea* tissue culture (Paper presented at ICAR. All India Second Workshop of AICIP on Medicinal and Aromatic Plants, held at Anand on November 1-4, 1976).

48. *CIMPO Newsl.*, 1975, **2**(4), 2-3.

49. Information from the Director, Tropical Products Institute, London.

50. Sarin, Y.K., Singh, A., Bedi, K.L., Kapur, S.K., Kapahi, B.K. & Atal, C.K. Observations on *Costus speciosus* Sm. as a source of diosgenin, *in* Cultivation & Utilization of Medicinal and Aromatic Plants, edited by Atal, C.K. & Kapur, B.M. (Regional Research Laboratory, Jammu-Tawi), 1977, 33-38.

51. Chakravarti, R.N., Mahato, S.B., Sahu, N.P., Pal, B.C. & Chakravarti, D. *Kallstroemia pubescens*—a new source for diosgenin, Part I. *J. Inst. Chem. India*, 1976, **48**, 170-73.

52. Puri, S.H., Jefferies, T.M. & Hardman, R. Diosgenin and yamogenin levels in some Indian plant samples. *Planta Med.*, 1976, **30**, 118-21.

53. Fieser, L. & Fieser, M. Steroids (Reinhold Publishing Corporation, New York), 1959, 831.

54. Khanna, P. & Jain, S.C. Diosgenin, gitogenin and tigogenin from *Trigonella foenum-graecum* tissue cultures. *Lloydia*, 1973, **36**, 96-98.

55. Khanna, P., Jain, S.C. & Bansal, R. Effect of cholesterol on growth and production of diosgenin, gitogenin, tigogenin and sterols in suspension cultures. *Indian J. Exp. Biol.*, 1975, **13**, 211-13.

56. Patel, C.B. A note on the oil from the fruit of *Balanites roxburghii. Curr. Sci.*, 1943, **12**, 58.

57. Ghanim, A.K. & Jayaraman, I. *Balanites roxburghii* as a source of diosgenin (Paper presented at International Symposium on Arid Zone Research Development, held at Jodhpur on February 14-18, 1978).

58a. Hardman, R. & Sofowora, E.A. A reinvestigation of *Balanites aegyptiaca* as a source of steroidal sapogenins. *Econ. Bot.*, 1972, **26**, 169-73; (58b) Isolation and characterization of yamogenin from *Balanites aegyptiaca. Phytochemistry*, 1970, **9**, 645-49.

59. Shah, C.S., Bhavsar, G.C. & Kapadia, N.S. Ontogenic variation of diosgenin in the leaves of *Balanites roxburghii* Planch (*B. aegyptiaca* Linn.). Proc. Second Workshop of AICIP on Medicinal and Aromatic Plants, held at Anand on November 1-4, 1976, 170-72.

60. Misra, G., Nigam, S.K. & Bhatnagar, S.C. Diosgenin from *Balanites roxburghii* leaves and kernels. *Indian J. Pharm.*, 1975, **37**, 103-04.

61. Vaughan, J.G. Structure and Utilization of Oil-Seeds (Chapman & Hall, Ltd., London) 1970, 255-56.

62. Jefferies, T.M. & Hardman, R. An improved infrared column-chromatographic quantitative isolation of diosgenin and yamogenin from plant crude extracts prior to their determination by infrared spectrophotometry. *Analyst*, 1976, **101**, 122-24.

63a. Hardman, R. & Wood, C.N. The ripe fruits of *Balanites orbicularis* as a new source of diosgenin and yamogenin. *Phytochemistry*, 1971, **10**, 887-89; (63b) The effect of ripening and aqueous incubations on the yield of diosgenin and yamogenin from the fruits of *Balanites pedicellaris. Planta Med.*, 1971, **20**, 350-56.

CHAPTER 3

EXTRACTION AND ASSAY PROCEDURES OF DIOSGENIN

Isolation, identification and estimation of diosgenin form important laboratory procedures for screening of plant materials, for working out economic feasibility of the source material for commercial extraction, it is necessary to find out the content of diosgenin as also the percentage which is economically recoverable. Extraction methods and assay procedures described here refer mainly to dioscorea tubers since these are of commercial importance.

Diosgenin (25 *R*-spirosta-5-en-3β-ol)* occurs generally in combined form as glycoside (saponin), and rarely in free state (1, 2ab, 3, 4). It is present as rhamno-rhamno-glucoside, called dioscin (Fig. 3.1), in tubers of commercial *Dioscorea* spp. (5). In *D. gracillima*, it occurs as glycoside, gracillin, with one rhamnose and two glucose units; and in *Trillium erectum* Linn. (Bethroot), as monoglucoside, trillin, and as diglucoside, trillarin (6ab). Presence of small amount of diosgenin in the form of its peptide ester with 4-hydroxy isoleucine has been reported in *Trigonella foenum-graecum* (7).

In free state, diosgenin is reported to occur in *Costus speciosus* (3) and *Kallstroemia pubescens* (4), and also in *D. deltoidea* from Kulu region (8). The enzymes saponases occurring in the plants are believed to hydrolyse the saponins into free diosgenin (4).

D. deltoidea tubers yield almost pure diosgenin (5, 9a). The genin appears to be present in different glycosidal forms. According to a recent work, one of them is a triglucoside, diosgenin-3-O-β-D-glucopyranosyl $(1\rightarrow3)$-O-{β-D-glucopyranosyl $(1\rightarrow4)$}-β-D-glucopyranoside (10). The full picture of the glucosidic pattern is yet to emerge.

D. prazeri tubers do not give as pure a diosgenin as *D. deltoidea* (9ab). Besides diosgenin, *D. prazeri* contains 3 sapogenins, prazerigenin-A {(25*R*)-spirost-5-en-3, 14-diol, m.p. 220-22°}, prazerigenin-B {(25*R*)-14-hydroxyspirost-4-en-3-one, m.p. 245-47°}, and prazerigenin-C {(25*R*)-14-hydroxy-5β-spirostan-3-one, m.p. 237-40°} (10, 11, 12). Diosgenin and prazerigenin-A are present as their rhamno-glucosides and rhamno-gluco glucosides. Prazerigenin-A is also present as monoglucoside (12, 10).

D. floribunda and *D. composita* tubers (Mexican yams) contain diosgenin (25*R*-), yamogenin (25*S*-) and pennogenin (17α-hydroxy

---

*In earlier literature, methyl group at 25*R* position has been referred to as 25α, and that at 25*S* as 25β.

derivative of diosgenin). It may be mentioned that as a raw material for the steroid industry, the epimers 25*R*- and 25*S*- are equally acceptable, singly and as a mixture. Pennogenin content may be 10 to 12 per cent of the total glycoside content; it is an impurity (5, 2).

The extracted diosgenin may be accompanied by sterols and sometimes by fatty oil (13).

Fenugreek (14) and *Balanites* spp. seeds (15-17) contain considerable amounts of both diosgenin and yamogenin unlike the dioscoreas where diosgenin predominates. In fenugreek, diosgenin is accompanied by a number of impurities.

EXTRACTION METHODS

Diosgenin can be extracted from the fresh or dried tubers by three methods, namely (i) Alcoholic extraction of the glycoside from the plant material, followed by acid hydrolysis, (ii) Acid hydrolysis of the total plant material, followed by extraction of the liberated sapogenin with a hydrocarbon solvent (18, 5), and (iii) Fermentation-cum-acid hydrolysis. Optimum conditions for the extraction of diosgenin have been the subject of intensive research.

Tubers dried in an oven at 80°C or in the sun and properly stored and handled under dry conditions do not lose diosgenin. Fresh tubers can be used if they are processed soon after harvesting, as there is a rapid decrease in diosgenin content when the rotting proceeds. It may be noted that *D. floribunda* tubers are extremely susceptible to rotting.

Alcoholic Extraction Method

This method, earlier employed by most of the diosgenin-producing firms in Mexico, is no longer in use. In this process dioscorea tuber chopped into small pieces, is air-dried at the collection centre and shipped to the extraction factories. Here it is finely ground and extracted twice with boiling ethyl or methyl alcohol. The filtered alcoholic extract is concentrated to a dark syrup, which is hydrolysed by heating with hydrochloric or sulphuric acid (2 to 4N) for a period of 2 to 12 hours. The precipitated crude diosgenin (about 85% pure) is recovered by filtration and washing with water. This material can be purified by crystallization from alcohol, chlorinated hydrocarbon, or hydrocarbon solvent (18).

Alcoholic extraction method gives frequently unsatisfactory results due to extraction of tuber pigments, the formation of gummy material and decomposition of unsaturated sapogenins to spirostadienes (19, 20, 21). Various modifications were adopted to avoid these drawbacks. One of the modifications, which had found wide use, was the treatment of the hydrolysed material with lime or other alkali to remove phenolic and other coloured impurities (18).

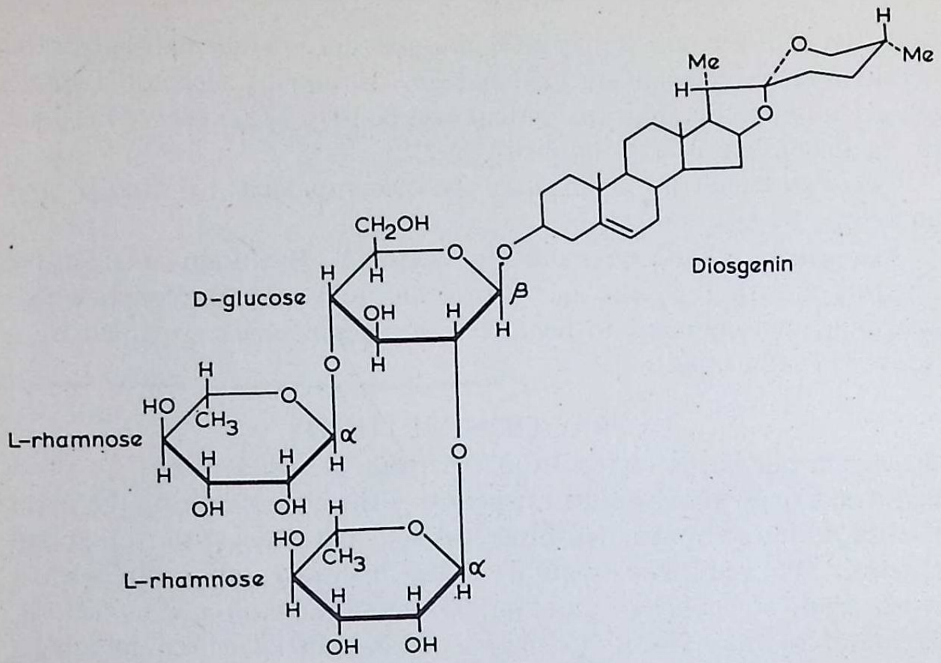


Fig. 3.1—Dioscin, a diosgenin glycoside

Peal (20) pointed out that during the acid hydrolysis of the extracted saponins, part of the available diosgenin (25*R*-spirosta-5-en-3*β*-ol) was lost by the formation of 25*R*-spirosta-3:5-diene due to dehydration in ring A and that higher the concentration of acid, greater the loss. According to Yamauchi (22), the diene formation was not only favoured by increasing the concentration of acid, but also by increasing the concentration of alcohol and the duration of heating. Of the three acids used for hydrolysis, viz. H_2SO_4, HBr and HCl, he found H_2SO_4 to be the mildest, and showed that refluxing dioscin (Fig. 3.1) with 4N H_2SO_4 for 2 hours was the optimum hydrolytic condition for releasing diosgenin.. However, purification of dioscin from the crude saponins in the alcoholic extract is not only tedious but requires a fairly high degree of manipulative skill. All these factors have made the alcoholic extraction method almost obsolete (19). Epimerization of the 25*R*- to 25*S*-form may also occur in the presence of ethanolic hydrochloric acid (23). Although this may not prove a real handicap for large scale processing, epimerization may affect the correct estimation of diosgenin on laboratory scale.

This method has one advantage in that it leaves the meal untouched, which can thus be used as a good source of starch (24a). Laevulinic acid can also be obtained as by-product.

Alcoholic extraction method is suitable for the extraction of diosgenin from fenugreek seeds or from *Balanites* seeds, where diosgenin will be available as a by-product precursor.

Acid Hydrolysis Method

This method, which involves releasing of sapogenins from their glycosides by hydrolysis *in situ*, not only saves time but also produces purer diosgenin; it is easier to handle, there being no tendency for gummy material to form at any stage. It has gained wide-spread use in India.

The process is a large scale adaptation of the method developed by Rothrock *et al.* (25), and consists of three main operations: preparation of the dioscorea tubers, hydrolysis of the saponin *in situ*, and extraction of diosgenin. The properly dried tubers are milled to 100 to 200 mesh or micropulverized, and the material is refluxed or heated in an autoclave with 2N to 4N mineral acids (preferably hydrochloric or sulphuric acid) for a period varying between 2 to 6 hours (18). Lower acid concentrations and shorter periods of hydrolysis are realized when the reaction is carried out under pressure. Thus, the same yield of diosgenin is obtained from either 0.2N hydrochloric acid slurry of the dry micropulverized tuber maintained at 150° under 3.87 kg/sq. cm pressure for 1 hour, or 2N solution refluxed for 2 hours at atmospheric pressure (25). In India, one of the firms uses 4N H_2SO_4 for hydrolysis of plant material in glass-lined steel reactors at 100°. However, optimum conditions for extraction—concentration of acid, temperature, pressure and heating time—may differ from manufacturer to manufacturer (9b).

The crude hydrolysate obtained on filtration is washed with water and limed till neutral, especially when sulphuric acid is used for hydrolysis. Insufficient washing of the hydrolysate before drying decreases the steroid content rapidly. The dried material is exhaustively extracted for about six hours with hydrocarbon solvent (e.g. petroleum ether, skellysolve B, skellysolve C, heptane), to yield diosgenin crystals. In India petroleum ether (b.p. 40-60°), hexane or toluene is used as the solvent. Diosgenin thus obtained is believed to be ideally suited for further processing to 16-DPA.

Blunden *et al.* (26) have reported that diene formation is minimized to 5-7 per cent of the sapogenin isolated, when hydrolysis is effected by refluxing the plant material with 2N HCl for 2 hours. Diene formation can be further reduced by aqueous incubation of the plant material before refluxing it under these conditions (17, 27); during incubation endogenous saponases partially or wholly hydrolyse the saponins to water-insoluble hemi-saponins or sapogenins which are less susceptible to acid dehydration at C-3 as compared to the water-soluble saponins.

The non-extractable matter does not contain any significant amount of starch. A high yield of laevulinic acid could be recovered from the acid liquor as a by-product (24a).

The Regional Research Laboratory, Jammu (28) has developed a process which has been covered by the Indian Patent No. 64,352 (1959). In this process, air-dried rhizomes of *D. deltoidea* are digested with boiling

5 per cent hydrochloric acid for 4 to 6 hours in an open pan. The hydrolysed product is filtered and washed free of acid. The residue is dried and extracted with light petroleum ether (b.p. 40-60°) in a continuous extraction apparatus for 10 to 12 hours. As the extraction proceeds diosgenin separates in the extraction flask as a crystalline product. The separated genin is filtered, and the filtrate is concentrated further to give another crop of diosgenin which is again filtered. From the mother liquor additional diosgenin is recovered by treatment with 2 per cent sodium hydroxide solution. The genin is washed with water to free it from alkali. The yield of diosgenin (m.p. 200-01°) is claimed to be about 95 per cent of the theoretical.

A process to recyclize the filtered acid (H_2SO_4) has been patented (29) in the U.S.A. In this process 100 g of dry dioscorea root material is refluxed for 4 hours at 96° with 300 ml of 4N sulphuric acid. The mixture is then cooled and filtered. The filtrate (150 ml) is used as recycle acid for further processing. A suitable series of extraction cycles is established in which such batch consists of 100 g of dioscorea root material and 4N sulphuric acid added to make up the volume in the batch having 150 ml of the recycle acid filtrate. The product from the series of acid treatment is washed, neutralized, filtered, and dried to give a series of brown residues of diosgenin.

The cake remaining after the extraction of diosgenin goes as waste; recyclization of this cake could be considered for economic recovery of certain products (9b).

Fermentation-cum-Acid Hydrolysis Method

The preliminary fermentation which involves enzymatic hydrolysis, reduces the weight of the material to be transported, for further hydrolysis by acid. It helps recover more diosgenin than any other method, and is being followed for commercial extraction of diosgenin in Mexico. The fresh, more or less whole tuber, or green root as it is called, is the usual raw material and is preferred to the sun-dried tubers, According to Hardman (5), Mexican Indian digs up the wild tubers and takes them to the local general store, there receiving payment for the tubers. Lorries tour these stores and fetch in the tubers to the fermentation centres, each one of which serves a radius of 20-30 km. At the processing centre, the green root is mashed in a hammer-mill and the resultant mash is put into a fermentation bin; the dried root is hammer-milled with water to produce a mash. It is then allowed to ferment for 2 days. Mash rises and the temperature inside the bin is then about 35-40°. Fermented mash is spread out on an open concrete area to dry in the sun. If it is raining, the mash is left in the bin. In sunny weather, it takes about one and a half days to reach a moisture content of about 7 to 8 per cent. The product is put into hemp sacks and sent to Mexico city, where it is hydrolysed with mineral acid at reflux temperature to release the diosgenin from its combined forms; the acid-free hydrolysed mass is extracted with heptane.

The addition of certain additives to the plant material during fermentation is said to improve the yield of diosgenin (5, 30).

According to Hardman (5), collection, fermentation and processing of the wild yams in Asia are similar to the Mexican process. This does not seem to be correct in the case of India as discussion with the indigenous producers of diosgenin revealed that they were purchasing only dry roots and using acid hydrolysis method. However, the dry tubers of *D. deltoidea* are soaked in water before hydrolysis by the CIMPO Drug Factory, Jammu (31).

This method may prove economical in otherwise uneconomical diosgenin-yielding plants such as *Costus speciosus* and *Kallstroemia pubescens*, where the content is below the accepted level for the purpose of acid hydrolysis method. The method is unsuitable where important by-products are to be obtained.

Other Methods

Incubation-cum-acid hydrolysis method—The method involves incubation of fresh plant material in water at 37°C for a period of a few hours to a few days before subjecting it to acid hydrolysis for obtaining diosgenin/yamogenin. This method is suitable for separating the sticky mesocarp of *B. aegyptiaca* fruit from its nut (32, 33a).

The method although not used commercially, is known to increase the yield of diosgenin, particularly from *D. floribunda* and *D. composita* tubers which are known to contain squalene (8-9 mg/100 g), a precursor of diosgenin. The increase observed could be due to the activity of certain micro-organisms associated with fresh tubers which may be converting the squalene to diosgenin. *D. deltoidea* rhizomes which contain a very small amount of squalene show hardly any increase (32). As stated under acid hydrolysis method, incubation reduces the diene formation, another plus point in its favour.

The findings are of interest since squalene, a petroleum fraction can be added to the plant material to obtain a better yield of diosgenin from the fresh tubers of *D. floribunda* and *D. composita*. The method can be used only near the dioscorea farm, since the usage of fresh material is of importance.

Good results can be obtained by another method which involves homogenizing of fresh root with an equal weight of water, adding of concentrated acid until the required strength is obtained, and extracting of the cake with a hydrocarbon solvent (18). This method is more suitable for analytical procedures.

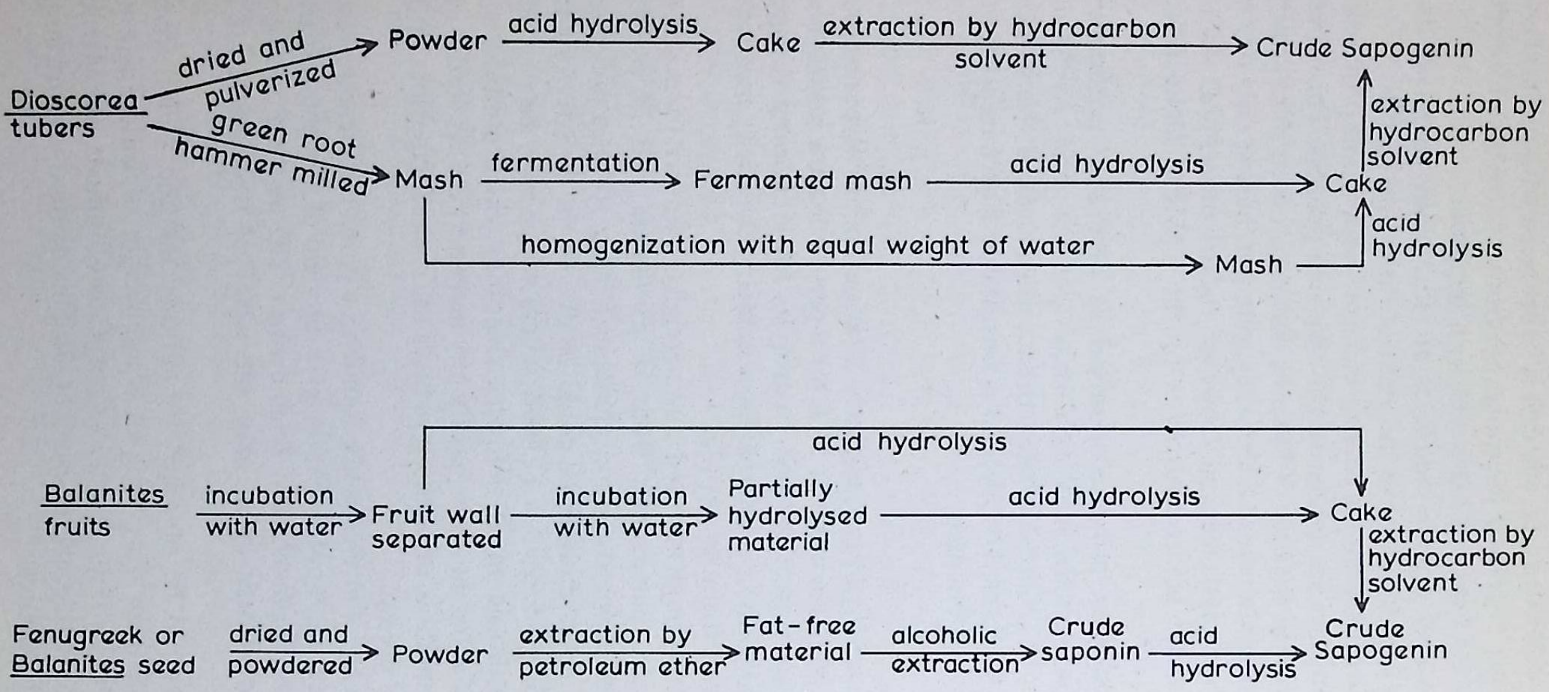

Dioscorea tubers
dried and pulverized
Powder
acid hydrolysis
Cake
extraction by hydrocarbon solvent
Crude Sapogenin
green root hammer milled
Mash
fermentation
Fermented mash
acid hydrolysis
Cake
extraction by hydrocarbon solvent
homogenization with equal weight of water
Mash
acid hydrolysis
Balanites fruits
incubation with water
Fruit wall separated
acid hydrolysis
incubation with water
Partially hydrolysed material
acid hydrolysis
Cake
extraction by hydrocarbon solvent
Fenugreek or Balanites seed
dried and powdered
Powder
extraction by petroleum ether
Fat-free material
alcoholic extraction
Crude saponin
acid hydrolysis
Crude Sapogenin

Fig. 3.2—Extraction methods for diosgenin from different sources

[Note: For *Costus speciosus* tubers and *Kallstroemia pubescens* the methods are same as those for *Dioscorea* tubers]

A method is being investigated to hydrolyse the saponin by exogenous enzymes, and extract it with a hydrocarbon solvent (9b).

The methods used for the extraction of diosgenin from dioscorea tubers, *Balanites aegyptiaca* fruit wall, seeds, and fenugreek seeds are summarized in Fig. 3.2.

The acid hydrolysis method is prevalent in India. The indigenous diosgenin-producing units operate mostly on pilot plant scale. Before the extraction on large or small scale, assaying of diosgenin is carried out from the tubers chosen at random for assessing the amounts of chemicals and solvents that would be required for extraction. In the case of cultivated source the tubers are excavated from the plants chosen at random in the field.

Machinery and Chemicals Required

No manufacturer of diosgenin likes to disclose the machinery, appliances and chemicals he uses. However, from the extraction processes it can be concluded that the following machinery may be needed for acid-hydrolysis method:

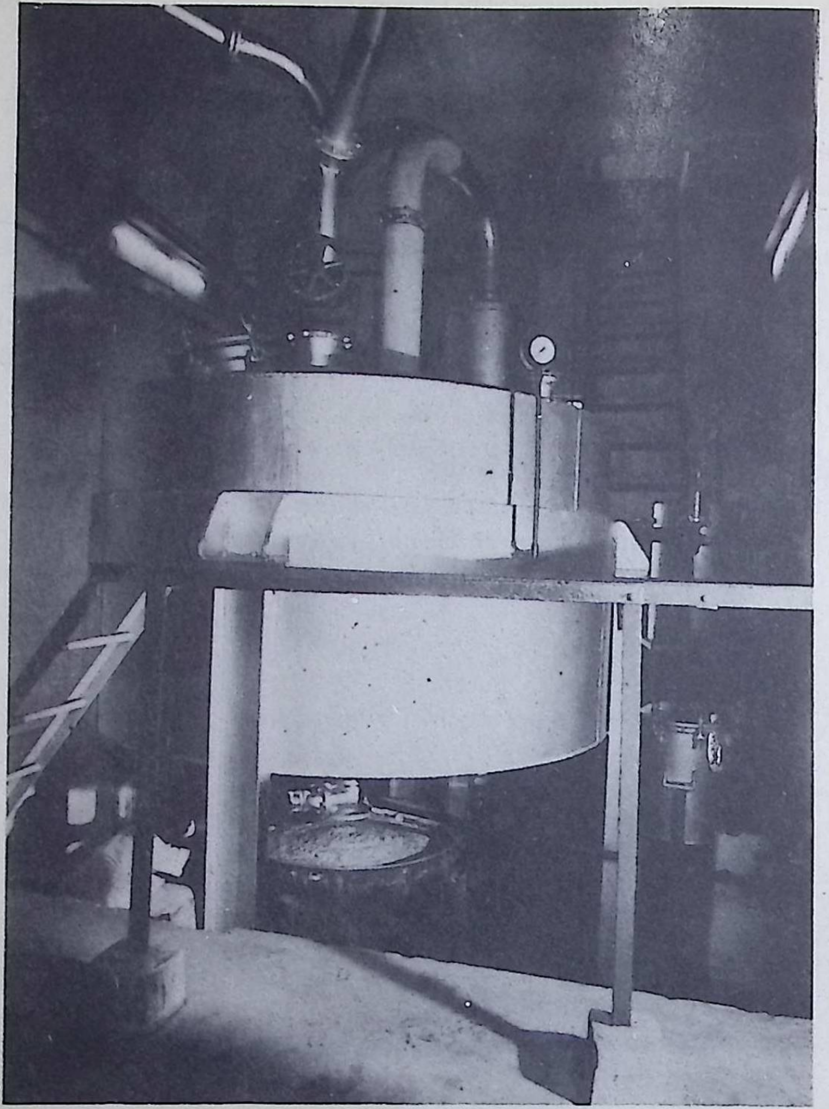
1. Micropulverizer or Grinder
2. Glass-lined steel reactors for hydrolysing the micropulverized tubers
3. Jacketed extraction vessel (see Fig. 3.3)
4. Jacketed concentration vessel, with packed column
5. Centrifuge
6. Oven and/or hot-air blower

The chemicals required are:

1. Hydrochloric or sulphuric acid
2. A small amount of additive like phenoxyacetic acid to promote hydrolysis (optional)
3. An alkali to neutralize excess acid
4. A non-polar solvent like hexane, toluene or petroleum ether for extraction

RRL, Jammu has recently introduced the latest technique of heat transfer—limpet coils instead of conventional jacket—as a result of which there is appreciable increase in the heat transfer coefficient, and consequent economy. The plant has been designed and installed at Mongpoo (Darjeeling) by RRL, Jammu, for the Directorate of Cinchona and Other Medicinal Plants of the Government of West Bengal.

Power and water connections are necessary. According to UNCTAD/GATT Report (38), the cost of installing a modern processing plant would



Dr Y.K. Hamied, CIPLA, Bombay

Fig. 3.3—Diosgenin extraction unit, CIPLA, Vikhroli, Bombay.

be approximately $ 2 million. In India, most of the manufacturers operate on pilot plant scale. The cost details of setting-up of a pilot plant for processing of diosgenin from dioscorea tubers are not available. However, according to one source the machinery cost comes to about Rs 0.4 million.

ECONOMICS OF DIOSGENIN EXTRACTION IN INDIA

Cultivated Tubers

According to Rabha (39) cost of extraction of one kilogram of diosgenin from 2-year old *D. composita* tubers by acid hydrolysis method comes to about Rs 500. Assuming that the cost of extraction from tubers of *D. floribunda* and *D. deltoidea* is the same as that from *D. composita*, the economics of diosgenin extraction from the cultivated *Dioscorea* spp. works out as presented in Table 3.1. The cost benefit analyses worked out for the three species are only approximates, since the actual processing costs will depend upon various factors such as percentage of recoverable diosgenin in the *Dioscorea* sp., and the hydrolytic conditions employed for extraction—the acid used (HCl or H_2SO_4) and its concentration, the temperature, and the time of heating. The purity of diosgenin required will also influence the cost and the sale value. It may be noted that the percentages of actual content of diosgenin and its economic recovery may differ. For example, about 2.5 to 3.0 per cent of diosgenin may be recovered economically from tubers containing say 3.0 to 3.5 per cent of the sapogenin (dry wt basis).

Table 3.1 shows that a cultivator-cum-extractor can make a net profit of Rs 43,000 to 59,000 per hectare from a 1-year old crop and Rs 62,000 to 69,000 from a 2-year old crop of *D. floribunda*, with the assumptions that extraction takes place near the site of dioscorea farm involving no transportation charges and that no interest is being paid for the total expenses involved. On these assumptions, the annual profit obtainable from 2-year old *D. floribunda* tuber is claimed to be much more than that from 2- or 3-year old *D. composita* or 3-year old *D. deltoidea* tubers. Processing of 3-year old crop of *D. composita*, with a yield of about 3.5 kg of dry tubers per plant and higher diosgenin content, improves the annual profitability (39). However, because of higher tuberization the plant density in the case of *D. composita* is thinner than that in *D. floribunda* and this partially offsets these advantages. Nevertheless, *D. composita* is a hardy plant withstanding vagaries of weather which is not the case with *D. floribunda*, while *D. deltoidea* in spite of its low tuberization is valued for its exceptionally pure diosgenin. Furthermore, close spacing of *D. deltoidea* will not only reduce the expenses but will also give higher yield of tubers and consequently higher yield of diosgenin. The profits can thus be improved from *D. deltoidea*.

The steroid drug companies which extract diosgenin are mostly located in Bombay area, whereas the dioscorea farms are located in Bangalore and Goa. This involves transportation charges for tubers, thereby increasing the cost of diosgenin extraction. However, CIPLA has set up diosgenin extraction unit in its factory in Bangalore, with a capacity of 36 tonnes of diosgenin per year (9b). Other cultivators might have followed suit. Also, the drug companies generally encourage *Dioscorea* cultivation by private farmers; the rate of cultivated tubers containing 3% diosgenin is about Rs 3/kg. Depending upon the percentage of diosgenin recovered, about 35-40 kg of tubers valued at Rs 105-120 (as in 1976) are

TABLE 3.1—ECONOMICS OF DIOSGENIN EXTRACTION FROM CULTIVATED DIOSCOREA TUBERS
(Approximates)*

	D. floribunda[1,2,*]		*D. composita*[3]		*D. deltoidea*[4,*]
	(1-year crop)	(2-year crop)	(2-year crop)	(3-year crop)	(3-year crop)
1. Dried tubers, tonnes/ha*	7.5-10.5	16.5-18.0	10.7	15.4	7.8
2. Diosgenin recovered, kg/ha	187-262 (2.5% recovery)	495-540 (3% recovery)	267 (2.5% recovery)	385 (2.5% recovery)	234 (3% recovery)
3. Cost of processing diosgenin (at the rate of Rs 500/kg)	93,500-1,31,000	2,47,500-2,70,000	1,33,500	1,92,500	1,17,000**
4. Cost of cultivation, Rs/ha†	12,500-19,000	24,500-24,000	22,500	27,990	17,000
5. Other expenses such as interest	—	—	24,000	24,000	1,000
6. Total expenditure for cultivation and processing, Rs/ha	1,06,000-1,50,000	2,72,000-2,94,000	1,80,000	2,44,490	1,35,000
7. Sale value of diosgenin (at the rate of Rs 800/kg in 1976-77)	1,49,600-2,09,600	3,96,000-4,32,000	2,13,600	3,08,000	1,87,200
8. Net profit from the crop‡	43,000-59,000	1,24,000-1,38,000	33,600	63,510	52,200
9. Annual profit/ha	43,000-59,000	62,000-69,000	16,800	21,170	17,400

[1]Bammi & Randhawa, Dioscorea Improvement Project—Status Report, 1972, 12-13; 1975, 52; [2]Bammi *et al.*, Extension Ser. No. 9, 1975 (IIHR, Bangalore; [3]Information from Shri L.C. Rabha, RRL, Jorhat; [4]Tajuddin *et al.*, *New Bot.*, 1976, 3, 44-47.

*Cost of diosgenin processing is applied uniformly to these species, however, it may vary with scale of operation, percentage recovery, hydrolytic condition, acids and solvents used.

**Interest for the amount has not been incorporated; with one year's interest, profit is marginal only.

†Cost of cultivation obtained by deducting net profit from the gross returns (see Table 2.7).

‡With the assumption that cultivator and processor are one and the same party and that processing is done near the site of the *dioscorea* farm.

Note: The cost of diosgenin processing could be as low as Rs 200/kg on large scale operation. The figures given here hold good for the year 1976-77.

required to extract 1 kg of diosgenin. The cost of extraction thus goes up to Rs 605 to 620 when the cultivator of tubers and the extractor of diosgenin are two different parties. Taking these factors into consideration along with the interest payable on investment, the profit estimates from diosgenin extraction should be much less than those indicated in Table 3.1.

The cost of diosgenin will obviously depend upon the content and actual recovery of diosgenin in the tubers used; higher the percentage of economic recovery, lesser the cost. Another factor which influences the cost is whether the method followed is acid hydrolysis or fermentation-cum-acid hydrolysis. In the latter process, mash weight will be much less and thus the amount of acid required will be accordingly less than that in the direct hydrolysis.

The economics of diosgenin depends greatly on the scale of operation. According to Dr Hamied (9a), if the price of the root is excluded, the cost of chemicals and processing charges for obtaining 1 kg of diosgenin from *D. deltoides* rhizomes containing 3 per cent diosgenin will come to about Rs 200 in large-scale operation. To this one has to add overheads, interest on investment, and depreciation to work out the actual cost. The cost figures for processing of *D. floribunda* tubers, according to the same source, are similar. The cost figures given by Rabha (39) for extraction of 1 kg of diosgenin from *D. composita* tubers (2.5% recovery), excluding the interest, appear to be comparatively high; but this refer to small scale operation.

Wild Tubers

The wild tubers of *D. deltoidea* are the major source of diosgenin in India. With a diosgenin content of 3.0 to 3.5 per cent, the quantity of tubers required to extract 1 kg of diosgenin will be about 30 to 35 kg valued at Rs 330 to Rs 385 at the rate of Rs 11/kg (as in 1976). Considering the expenditure on extraction of 1 kg of diosgenin as Rs 500, the total cost of diosgenin comes to between Rs 830 and Rs 835 per kilogram. It explains the high selling prices of Rs 1100 and above per kg diosgenin from wild *D. deltoidea* tubers in 1976. The wild rhizomes are now selling at Rs 6 or 7 depending upon the quality, and the prices of diosgenin are now between Rs 500 to 750 (9a).

ISOLATION AND IDENTIFICATION

Whether it is dioscorea tuber or a plant sample to be screened for the presence of diosgenin, whether it is cell mass from *in vitro* culture (tissue culture) or a plant part from a breeding programme, the method of choice for the isolation of diosgenin in the laboratory is the acid hydrolysis method.

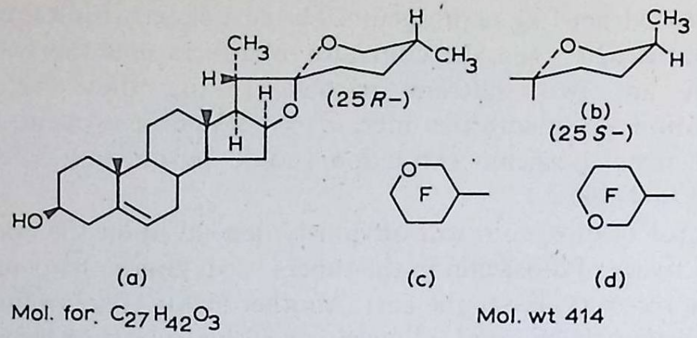


Fig. 3.4—Structure of diosgenin (25*R*-): (a) Stereospecific structure with conventional ring F as a chair; (b) 25*S* position in yamogenin; and (c) & (d) Ring Fs drawn simply.

TABLE 3.2—PROPERTIES OF DIOSGENIN AND YAMOGENIN[1,2]

Properties	Sapogenin Diosgenin (25*R*-)	 Yamogenin (25*S*-)	Derivative Diosgenin acetate	 Yamogenin acetate
Mol. for.	$C_{27}H_{42}O_3$	$C_{27}H_{42}O_3$	—	—
Mol. wt	414	414	—	—
m.p. °C (*P*-TLC)	208-09 (204-08)*	187-8 (184-85; 201)*	193-94 (192-98)*	172-3 (182)*
$[\alpha]_D^\circ$ (CHCl$_3$)	-127 ± 3 (-129)*	-150 ± 3 (-129)*	-127 ± 3 (-121)*	-116 ± 3 (-119)*
Characteristics IR bands	1240, 1160, **980**, 960, **900**, 865, 795, 670, 620, 595 cm^{-1}	1270, 1210-25, 1130, 1060, 990, **980, 920**, 850, 680, 610, 585 cm^{-1}		
Solubility	Soluble in all organic solvents and acetic acid	More soluble in all organic solvents and acetic acid and in fatty oil containing liquors than diosgenin		

[1] Jefferies & Hardman, *Planta Med.*, 1972, **22**, 78-87; [2] Dictionary of Organic Compounds, 4th ed., 1965, Vol. 3, 1262.

*Figures in parentheses show the range of values reported in literature including Dictionary of Organic Compounds (1965) and Rodd's Chemistry of Carbon Compounds (1971).

Extracted diosgenin can be purified by repeated crystallization, or by preparative thin layer chromatography (*P*-TLC) or by column chromatography.

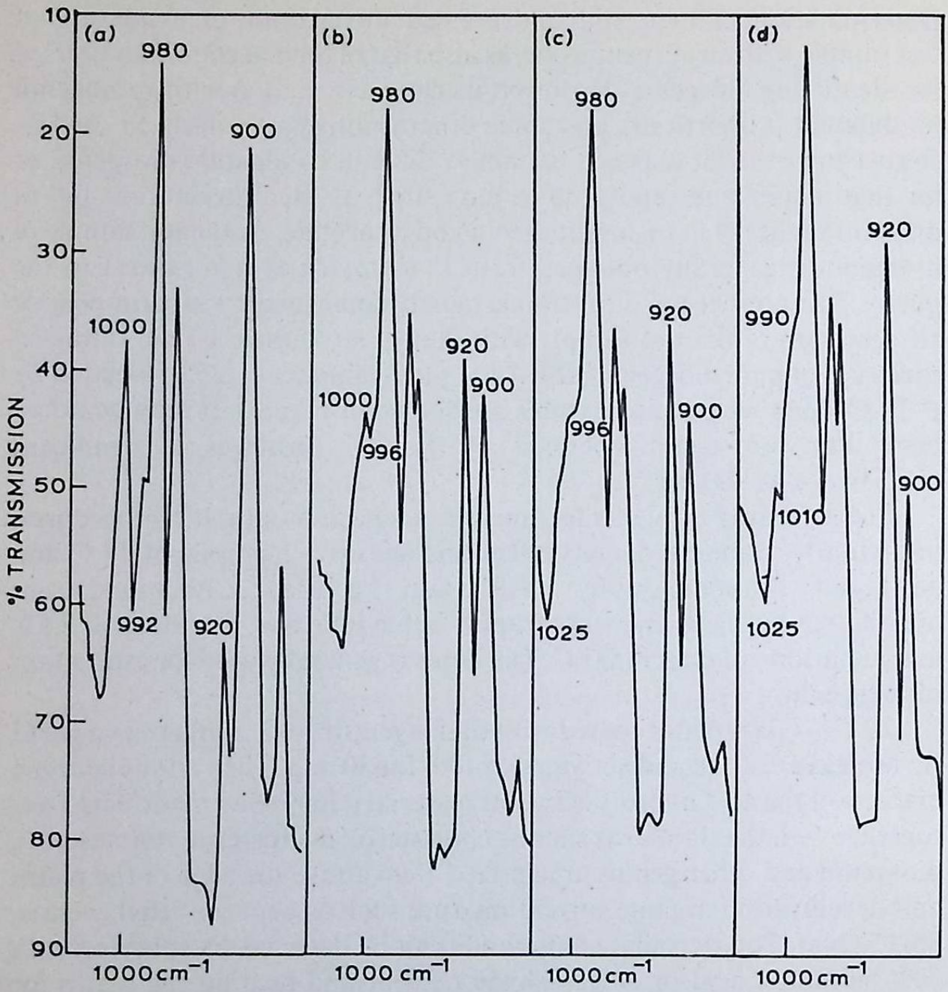
Properties of diosgenin (Fig. 3.4) and its 25*S*-epimer, yamogenin, with which it is invariably associated are summarized in Table 3.2.

Determination of m.p. and undepressed mixed m.p. of a mixture of test sample with an authentic one, as also that of their acetates can be used for identifying diosgenin. However, its close association with yamogenin (in different proportions), and some other genins particularly 5α- or 5β-, sterols and even fat makes it extremely difficult to separate diosgenin or for that matter any sapogenin in pure state; as such literature is full of different ranges of m.ps. for diosgenin and its acetate. Authentic sample of diosgenin is generally obtained from *D. deltoidea* as it is nearest to the purity. The presence of diosgenin is mostly confirmed by superimposible IR spectrum of the test sample with that of an authentic one. Infra-red spectrum of pure diosgenin (25*R*-) and pure yamogenin (25*S*-) isolated by *P*-TLC along with their mixtures are shown in Fig. 3.5. It may be noted here that yamogenin obtained by *P*-TLC possesses a significant absorbance at 900 cm^{-1}.

For screening of plants for diosgenin, chromatographic procedures, particularly more rapid, convenient and sensitive methods of TLC and gas-liquid chromatography (GLC) can be used. Compounds are identifiable by their chromatographic behaviour, viz. R_f values in TLC and retention volumes in GLC. The latter is generally used for estimation of diosgenin.

TLC—Glass plates coated with thin layers (0.2-0.3 mm) of silica gel G (E. Merck) are dried and activated at 100° for 30 min. The various benzene extracts of the acid hydrolysed plant material (after being made acid-free) together with the standards such as cholesterol, β-sitosterol, stigmasterol, diosgenin and other genins are applied 1 cm above the edge of the plates and developed in organic solvent mixture such as benzene-ethyl acetate, 85:15 (34a). The steroidal compounds can be detected by spraying with 50% sulphuric acid or anisaldehyde reagent and heating the plates for 10 minutes; with the former reagent, diosgenin gives greenish brown spot, while with the latter, a green spot (R_f 0.42). The coloured spot is distinct from those of the sterols; β-sitosterol and cholesterol give reddish pink colour with sulphuric acid (50%) while stigmasterol gets resolved to greyish violet spot; with anisaldehyde the three sterols migrate as single violet zone (35a).

P-*TLC*—Silica gel G thick layer (0.4-0.5 mm) glass plates are activated and benzene extract applied along with the reference standard diosgenin. The unsprayed plates are visualized under UV light; and the band which corresponds to standard reference, is marked. Various marked bands from 200 plates are collected and extracted with benzene. Part of the isolated compound is recrystallized and m.p. and mixed m.p. with an authentic sample determined. Other part of the isolated compound is acetylated, chromatographed and again collected. The melting point of diosgenin acetate (193-94°) further substantiates the presence of



Jefferies & Hardman, Planta Med., 1972, 22, 83

Fig. 3.5—Infra-red spectra 1% W/V solutions in chloroform in a 1 mm cell of (a) Pure diosgenin; (b) Diosgenin: yamogenin mixture 1:2; (c) D:Y as 1:3; and (d) Pure yamogenin.

diosgenin. IR spectrometry of both diosgenin and diosgenin acetate with standard references is used for confirmation.

Typical examples of detection (isolation and identification) of diosgenin by TLC are provided by several workers (34ab, 35a, 36a, 37).

Diosgenin-yamogenin pair can be resolved from tigogenin-neotigogenin pair by column chromatography using silver nitrate impregnated alumina (15%) as adsorbent and the solvent benzene-petroleum ether (2:1), (2:3). The method is efficient for the separation of Δ^5-sapogenins from their corresponding saturated compounds with which they occur in nature, for example, in fenugreek seeds (37).

ASSAY PROCEDURES

Several analytical procedures are available for the estimation of diosgenin (and/or yamogenin) for commercial purposes. Among them are the gravimetric (25, 40-44); colorimetry (45, 46, 13); gas liquid chromatography (GLC) (47, 48); densitometric thin-layer chromatography (TLC) 26, 49); infrared spectrometry (IR) (19); and column-chromatography-IR spectrometry (36b).

Assay procedures are described here with particular reference to commercial dioscoreas and fenugreek seed which is being established as a commercial source in Morocco and possibly in some other countries. The relative merits and demerits of various assay procedures for diosgenin are summarized in Table 3.5. A perusal of the table shows that all the methods suffer from one drawback or the other and it has been, therefore, necessary to use the combination of methods such as column chromatography (36b) or preparative TLC (*P*-TLC) with IR-spectrometry (36a) or *P*-TLC with colorimetry (27) to obtain with precision the content of diosgenin in the plant material; even in combination, the result may not be perfect. However, this precision may not be necessary for commercial *Dioscorea* spp. since manufacturers use diosgenin of about 90 per cent purity. *D. deltoidea* tubers contain almost pure diosgenin and any assay procedure is as good as any other. In the case of *D. floribunda*, *D. composita* and *D. prazeri*, sophisticated methods may be employed. For fenugreek and *Balanites* seeds combination methods are a must, because here a fair degree of precision is needed especially as estimation of yamogenin with IR spectrometry alone leads to inflated figure in the presence of a fatty oil (36b).

The gravimetric methods for determining diosgenin in yams require 50 g or more of tuber for each determination. The method as such is termed macro-procedure and is essentially a large scale and more quantitative isolation (50). The other methods listed above are micro-procedures and require from 10 g to as small as 50 mg of plant material. Micro procedures can be used at a much earlier stage of plant growth, effecting considerable saving of time in a breeding programme or at various stages of cell-growth in tissue cultures.

Of the several methods available, the extractors of diosgenin from dioscorea tubers generally use gravimetric procedure along with a micro procedure depending upon the facilities available in the laboratory. Each manufacturer makes sure that purity of diosgenin is 90 per cent or above. Purity can be judged by subjecting the assayed diosgenin (by gravimetric procedure) to TLC or column chromatography. *D. floribunda* and *D. composita* yield diosgenin (+yamogenin) of about 90 per cent purity. In case the purity is below the accepted level, diosgenin is purified by fractional crystallization. For fenugreek, gravimetric procedure may not be of help and combination of microprocedures is adopted.

Gravimetric Methods

Gravimetric methods include those developed by Morris *et al.* (40) and Rothrock *et al.* (25), or their modifications.

Morris *et al.* reported that the comminuted sample slurry of *dioscorea* tubers when hydrolysed with about 2N HCl for 3 hours yielded maximum diosgenin. The method as modified by Selvaraj (44) is used in India for assaying diosgenin by one manufacturing firm and is described below.

The tubers washed with water and subsequently dried in shade are used for assaying purposes. The moisture content in the sample is determined by drying the powdered material in an oven for 6-8 hours at 100°. About 20 g of the material is blended thoroughly in a 'Braun' mixer with 50 ml of water for 5 minutes. The slurry is transferred to a 500 ml flask containing 150 ml of water and a calculated amount of 11.3 N HCl added to maintain the required acid concentration of 2.5N. The flask fitted with a condenser is placed on a boiling water-bath for 2 hours to complete the hydrolysis. The slurry after hydrolysis is allowed to attain room temperature and filtered in a Buchner funnel under vacuum. The residue is washed with distilled water till the filtrate is free from acid. The residue along with the filter paper is transferred to a petri-dish and dried in an oven at 100° for 6 hours. It is then extracted with petroleum ether (b.p. 40-60°) in a Soxhlet apparatus for 8 hours. The extracted solvent containing diosgenin is concentrated to about 25 ml, chilled in ice and filtered. The mother liquor obtained after filtering and washing diosgenin is concentrated, chilled in ice and a second crop of diosgenin, if any, is added to the first crop and the whole diosgenin weighed after drying it in an oven for 2 hours at 100°. The purity of the crude diosgenin extracted by this process is found to be 86.8 to 87.6 per cent of total sapogenin as shown by crystallization studies.

Rothrock *et al.'s* method (25) consists of hydrolysis of finely ground tuber (fresh or dried) with 2N HCl at boiling temperature for 2 hours and extraction of the acid-free dry hydrolysate in a Soxhlet apparatus using petroleum ether (b.p. 40-60°) for 6 hours.

Chaudhuri (51) of Regional Research Laboratory, Jorhat has investigated four different gravimetric methods (25, 42, 43, 44) of analyses of diosgenin in *D. floribunda*, *D. composita*, and *D. prazeri*. According to him, although all the four methods gave almost identical yields of diosgenin, the method standardised by Rothrock *et al.* was less time-consuming and gave whiter product, with sharper and better melting points before purification of diosgenin. The purity of crude diosgenin obtained by this procedure was found to be 91.0, 87.4 and 95 per cent in the case of *D. floribunda*, *D. composita* and *D. prazeri* respectively, as determined by column chromatography.

With a view to optimizing the conditions of hydrolysis of tuber, Chaudhuri carried out experiments by varying the acid concentration, hydrolysis time and also solid to liquid ratios. *D. composita* and *D. floribunda* tubers were used in the studies. Hydrolysis of fresh or dry samples with 2N HCl in a ratio of 1:18 at the boiling temperature for 2 hours gave maximum yield of diosgenin. It may be mentioned here that Chaudhuri has not taken into consideration the hydrolytic conditions used by Brain *et al.* (19) for isolating diosgenin.

For merits and demerits of gravimetric and other assay procedures, see Table 3.5.

Colorimetric Methods

The colorimetric methods of assaying diosgenin uses the principle of selective red or pink colour formation of Δ^5-sapogenins (spirosta-5-en) with antimony ions.

Yamagishi & Nakamura (45) developed a colorimetric procedure for the estimation of pure diosgenin by a solution of antimony trichloride ($SbCl_3$) in a mixture of nitrobenzene and methanol; the solution requires heating for at least 20 minutes at 60° to develop the colour. Sofowora & Hardman (13) have developed a spectrometric method for the estimation of diosgenin in crude plant extracts. They have used the method of Brain *et al.* (19) for extraction of diosgenin from the plant material, which takes more than 40 hours to obtain crude plant extracts. The stability of the red colour varies with time and reagents.

A rapid spectrophotometric method for the quantitative determination of diosgenin from dioscorea tubers, recently developed by Rishi *et al.* (46) is described below.

Preparation of standard curve—Diosgenin was isolated from *D. deltoidea* and purified by preparative TLC, 24% $SbCl_5$ solution in 70% $HClO_4$ was used for detection. 25 Mg of pure diosgenin were dissolved in 250 ml of hexane (solution A) and 1.0 ml of this solution was taken in two tubes and the solvent removed by bubbling air. $HClO_4$ (5 ml) followed by 0.1 ml of 24% $SbCl_5$ solution prepared in $HClO_4$ (70%) were added to each tube. The mixtures were kept for half an hour. Absorbance was noted for both the solutions from 400 to 600 nm against reagent blank. A maximum absorption was noticed at 486 nm.

Volumes corresponding to 10, 20, 30, 40, 50, 80 and 100 μg of diosgenin were taken from solution A into different tubes, solvent removed and colours developed as mentioned above. The absorbance of each was noted at 486 nm against reagent blank. A straight line was obtained when the concentration of pure diosgenin (10, 20, 30, 40, 50, 80

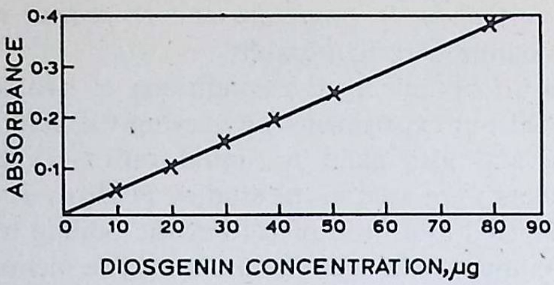


Fig. 3.6—Standard diosgenin curve for colorimetric determination.

and 90 μg) was plotted against absorbance (Fig. 3.6). The colour reaction follows Beer's Law at room temperature.

Hydrolysis and extraction—Finely powdered tuber of *D. deltoidea* was dried at 105° and passed through 72-mesh sieve. 50 Mg of this material and 25 ml each of 3N HCl and hexane were refluxed in an all-glass apparatus on a magnetic stirrer hot plate for 2 hours at 90-96°. The mixture was allowed to cool and the aqueous phase was given two shakings with 25 ml of hexane each time and the combined organic phase was washed with 1% $NaHCO_3$ solution and subsequently with distilled water and the volume made to 100 ml with hexane. From suitable aliquots of this extract (usually 1 ml) solvent was removed and colour developed as described above and the absorbance measured at 486 nm against the reagent blank. The concentration of the unknown sample was read from the calibration graph corresponding to its absorption and the percentage of diosgenin calculated in *D. deltoidea* rhizomes. The accuracy and efficiency of the method was judged by undertaking gravimetric analysis using the procedure of Morris *et al.* (40). The results were within the limits of experimental error. Only 50 mg of the dried material is required for the estimation. Both hydrolysis and extraction can be completed in about 2 hours. The quantitative aspect of the extraction has been confirmed by TLC of the aqueous layer. Hydrolytic conditions were thoroughly checked to make certain that the release of diosgenin was complete. In order to speed up the assay, advantage was taken of the immiscible character of hexane with aqueous phase. Hexane was found to be the most suitable of all solvents tried.

The method has an edge over the method of Sofowora & Hardman (13) in that it is very rapid; 8 to 10 samples can be assayed by one analyst in one day. The other distinguishing features of the method are:

(a) small quantity (50 mg) of the dried plant material required
(b) hydrolysis and extraction achieved in 1½ to 2 hours,
(c) avoids additional solvents, and
(d) the use of smaller quantities of antimony ion for the colour reaction.

GLC Methods

GLC methods involve preparation of a column of a calibration graph, and of the sample to be assayed. Two procedures for assaying dioscorea tubers, one developed by Cooke (47) and the other by Dixit *et al.* (48) are described here.

In Cooke's method, a Wilkens Aerograph Gas Chromatograph, Model 205-2B, with flame ionization detector was used.

Preparation of column—A pyrex glass column, 30 inches × 1/8 inch, was packed with acid-washed DMCS-treated Chromosorb G (80-100 mesh) coated with S.E. 30. This was done by adding a 2.5 per cent hexane solution of S.E. 30 to acid-washed DMCS-treated Chromosorb G in a beaker, swerling the mixture, and allowing it to stand for 2 to 3 minutes. The volume of S.E. 30 solution was sufficient to cover the Chromosorb G and enable a slurry to be made. The slurry, after standing, was filtered through a Whatman No. 1 paper on a Buchner funnel under suction. After air drying, the coated support was transferred to a watch-glass and oven-dried at 100°. The column, after packing, was matured for 48 hours at 260°.

Operating temperatures were 240° for oven and injector block, and 250° for detector. Gas flow-rates were 28 ml/minute for nitrogen, 26 ml/minute for hydrogen and 250 ml/minute for air. A typical chromatogram of diosgenin from *D. deltoidea* extract is shown in Fig. 3.7.

Preparation of calibration graph—A range of standard solutions was prepared by dissolving the recrystallized diosgenin (m.p. 205-06°) in redistilled 2-ethoxyethanol. The lowest practical standard was about 0.2 μg/μl due to detector sensitivity to the solvent, and the highest standard was limited to about 10 μg/μl by the solubility of diosgenin in the solvent.

Response of detector was checked by injecting 1 μl of a range of standard solutions of diosgenin up to 10 μg/μl. The plotted peak heights measured at or converted into the same attenuation (setting 4) gave a straight-line graph as shown in Fig. 3.8.

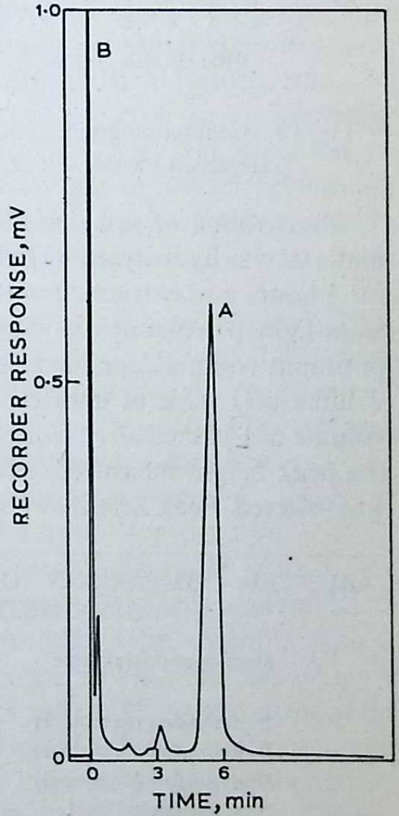


Fig. 3.7—Gas chromatogram of diosgenin from *D. deltoidea* yam extracts.

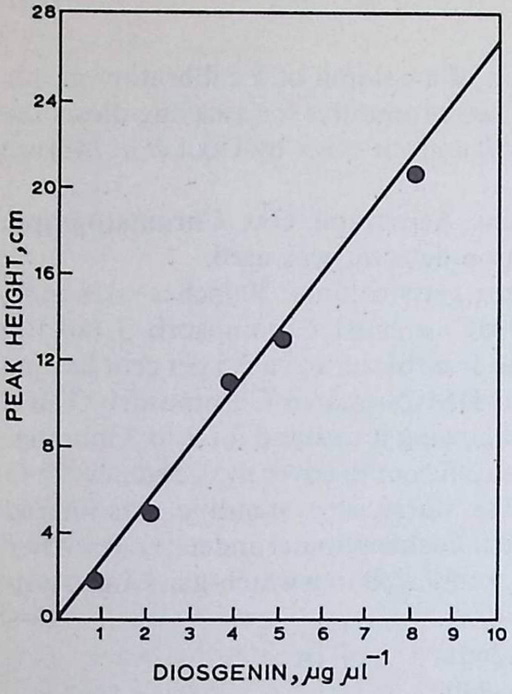


Fig. 3.8—Calibration graph of diosgenin (injection volume = 1 μl).

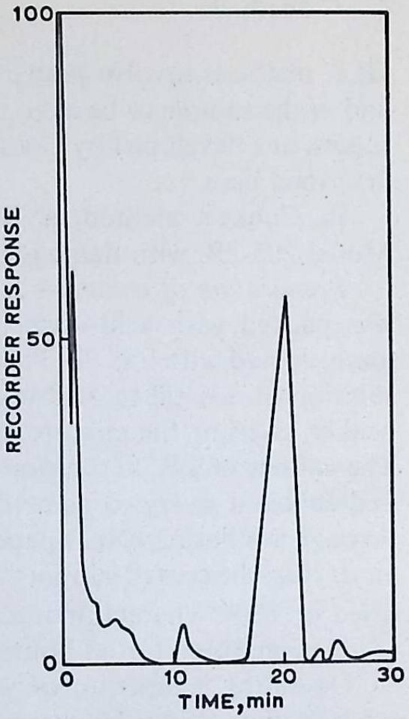


Fig. 3.9—Gas chromatogram of *Dioscorea floribunda* yam extract.

Preparation of sample—The dried sliced tubers or undried minced material was hydrolysed by refluxing with 2N HCl (10 ml/g of dry sample) for 3 hours and extracting with light petroleum (60° to 80°). The residue from light petroleum was dissolved in redistilled 2-ethoxyethanol. The optimum volume depended upon the amount of diosgenin present but a volume of 1 ml/g of undried tuber used could be taken as a guide. A volume of 1 μl measured from a Hamilton 701N syringe was inserted and the peak height measured. From this an appropriate standard solution was selected. Peak heights were compared by measuring in the usual way.

TABLE 3.3—COMPARISON OF VALUES OBTAINED BY GLC AND GRAVIMETRIC DETERMINATION[1]

Homogenous sample	GLC method	Gravimetric method
D. deltoidea (sample 1)	2.9	2.8
D. deltoidea (sample 2)	5.5	5.6
D. deltoidea (sample 3)	5.5	5.1
D. sylvatica	4.1	4.0

[1] Cooke, *Analyst*, 1970, **95**, 95-97.

Validity of the method was checked by comparing the values obtained by chromatographic determination and those obtained by gravimetrically. They were comparable as shown in Table 3.3.

Dixit *et al.* (48) of National Botanic Gardens, Lucknow and Central Drug Research Institute, Lucknow, have developed a GLC method using a Varian Aerograph Model 1868-4 with flame ionization detector. A stainless steel column, 60 inches × 1/8 inch, packed with chromosorb W (80 to 100 mesh) coated with 3 per cent S.E. 30, was employed. The operating temperature of the oven was 235°, of the injector block 250°, and of the detector 250°. It was observed that the column was quite stable. The flow rate of nitrogen, the carrier gas, was 45 ml/minute, of hydrogen 30 ml/minute, and of oxygen 250 ml/minute. Under these conditions, the retention time was 20 minutes. A typical gas chromatogram of *D. floribunda* yam extract (1 μl aliquot) is shown in Fig. 3.9.

A calibration curve was obtained from GLC of aliquots of a solution containing diosgenin (5 mg) in methylene chloride (1 ml) by plotting the concentrations against the peak areas in the GLC. The peak areas can be measured and checked by triangulation method (35b). The curve was linear. Diosgenin content of unknown samples were then obtained from areas of the diosgenin peak in the GLC from the calibration curve.

Diosgenin from the dioscorea tubers was also extracted by Selvaraj's gravimetric method. The results obtained by GLC were somewhat less as diosgenin alone is estimated by this method, while in Selvaraj's method, other constituents that come in the diosgenin extract are also weighed.

Densitometric TLC Procedures

Diosgenin generally occurs in plants along with yamogenin. This procedure can separate one pair of 25*R* and 25*S* from their dienes and from the sterols, etc. found in the crude plant extract; unlike the infra-red assay, the method fails to separate individual epimers. The TLC method has been made quantitative by means of densitometry. Blunden *et al.* (26) used densitometric TLC for the quantitative estimation of diosgenin. Brain & Hardman (49) improved upon the spotting procedure.

Blunden *et al.* employed TLC to separate diosgenin from other constituents present in dioscorea tuber extracts. Diosgenin was rendered visible by $SbCl_3$ and the coloured spot estimated by scanning with a photoelectric densitometer attached to an integrating logarithmic recorder. The procedure has an error of about 7% when applied in concentrations between 10 and 75 μg of diosgenin. Assay results obtained for diosgenin by this method were slightly lower than those obtained by conventional IR spectrophotometry, since the 25 *R*-spirosta-3, 5-diene formed during the extraction of diosgenin was not estimated in the chromatographic method.

Vitatron densitometer was used by Blunden *et al.* A linear relationship was obtained between the logarithm of the weight of sapogenin applied to the plate and the square root of the absorbance.

One of the major sources of error in quantitative TLC is the spotting procedure. Brain & Hardman prepared a template and syringe assembly for sample application which improved the precision of quantitative determination. This assembly is used for determining diosgenin in dioscorea tubers on silica gel G plates. The procedure is accurate to ± 2% and is particularly useful for assaying sample in breeding programmes where precision is so important. The method is extremely rapid, only 15 seconds were required for five spots of one solution. The plates were developed for 25-30 min in 8:1 hexane-acetone and the sapogenin spots were charred with 300 g antimony trichloride in 100 ml HCl before scanning. The relative standard deviation was 1.1% for 5 μg of sapogenin. A direct relationship was found between the weight of sapogenin applied and the integrated absorbers reading over the range 4.5 to 6.5 μg of diosgenin. The relationship between integrator reading and sapogenin concentration is not direct and a correction curve was drawn up for the use with a 5 μg standard and unknown readings assuming a direct relationship, and this was then correlated with the absolute concentration by means of the correction graph and expressed as a percentage of the original plant material on a moisture free basis.

The sapogenin was isolated in the manner described by Brain *et al.* (19) but the final residue was dissolved in sufficient chloroform to give a total sapogenin concentration of approximately 0.1 per cent.

IR Spectrophotometry

For the rapid routine quantitative screening of plant material infrared spectroscopic methods have been used based on the characteristic spirostan structure of the sapogenin. Wall *et al.* (21) are the pioneers in this field; however in their alcoholic extraction method followed by hydrolysis by 4N HCL, a portion of diosgenin was lost by the formation of 3:5-diene and their total estimation of diosgenin included this diene.

Brain *et al.* (19) have extended the method of Wall *et al.* to the estimation of diosgenin and its 25 *S*-epimer, yamogenin, and improved by using the preferred procedure of extraction of sapogenins involving acid hydrolysis *in situ.*

Isolation of crude sapogenin—A known weight (1-10 g) of the sample of plant material, of known moisture content, in whole or in powdered form is hydrolysed by heating for 2 hours under reflux with 2N HCl, using 20 ml/g of dried plant material. After cooling and filtering the mixture, the acid-insoluble residue is washed with water (20 ml/g), before neutralization with 40 ml/g of 5 per cent w/v ammonia solution.

The insoluble residue, after it is drained, is dried in a hot-air oven at 60° for 16 hours before it is extracted in a Soxhlet with light petroleum (b.p. 40-60°) to exhaustion; extraction normally takes 24 hours. The solvent is removed in a rotary vacuum evaporator and the residue dissolved in chloroform to give a total sapogenin concentration of 0.5 to 1.0 g per 100 ml.

Estimation of sapogenin—The spectrum of the crude sapogenin solution is run over the 1000-800 cm^{-1} region. The trace is inspected for the presence of the four or five infrared spectrum bands which are characteristic of the spirostan structure. For estimation, see Recommended calculation method under *P*-TLC—IR Spectrometry.

The quantitative estimation can be made of 25*R*- and 25*S*-sapogenins individually and together in crude plant extract. The method is rapid and has an overall error of 3-10 per cent for individual C-25 epimers and 3-5 per cent for sapogenin mixture. The procedure is appropriate for the study of the variation in C-25 epimers in morphological parts and with season, as well as for routine screening of steroidal sapogenins of economic importance. It can be used for *D. deltoidea* without any appreciable error but the percentage error may be more in determining sapogenin content of *D. floribunda* and *D. composita.*

Brain *et al.* used diosgenin (25*R*-) and sarsasapogenin (25*S*-), the latter because yamogenin (25*S*-) was not available. Jefferies & Hardman (36a) have separated the two epimers diosgenin and yamogenin by *P*-TLC and thereby improved the infra-red estimation of Brain *et al.*

P-TLC—IR Spectrophotometry

The method used by Jefferies & Hardman (36a) essentially consists of obtaining the crude sapogenin extract, isolating diosgenin (D) and yamogenin (Y) by *P*-TLC, and subjecting the sapogenins individually and in mixtures in different proportions to IR spectrophotometry and estimate the D + Y and D:Y ratio.

D and Y from *T. foenum-graecum* seeds were obtained by using acid hydrolysis method; those from *B. aegyptiaca* root by using alcoholic extraction method. Petroleum ether extract of the former is rich in D, while that of the latter is rich in Y. In common solvents yamogenin is more soluble than diosgenin and fractional crystallization from acetone or petroleum ether (b.p. 40-60°) was used to afford diosgenin- or yamogenin-rich fraction prior to *P*-TLC.

P-*TLC procedure*—The separation was at first carried out using Shandon continuous elution tanks and 20 × 20 cm plates. Adsorbent (silica gel G), 75 g, with distilled water 165 ml, was spread over five plates at 1 mm thickness, left overnight prior to activation at 110° for 1 hour. The separation obtained by Bennett & Heftmann's method (52) using dichloromethane: diethylether, 199:1, for 6 hours is inadequate for preparative purposes but a distinct zone between the epimer bands was achieved with dichloromethane: diethylether, 196:4 for 16 hours and a sample loading of 25 mg per plate. The epimers were detected at 366 μm as pale blue fluorescence on a yellow background. The sample was applied in chloroform using a Desaga mechanical applicator, which gave origin bands of less than 1 cm wide when solvent evaporation was assisted by hot air.

The material was scraped off and then eluted from the adsorbent by Soxhlet extraction using methanol for 24 hours and the sapogenin assayed by IR analysis for purity; this showed a trace of a minor sapogenin, which was removed by a second *P*-TLC separation and crystallized from acetone or 85% aqueous methanol. Recovery of pure sapogenin was 69 per cent and weights of individual sapogenins were in the same ratio as the IR analysis of the starting material. Purity was not improved by third *P*-TLC.

Another method of band collection was developed to avoid the narrow zone between the two epimer bands. The extracted sapogenins were then as pure as those obtained by two *P*-TLC separations; the overall yield was 60%. Gitogenin, which is also present in fenugreek seeds gets separated from the monohydroxy sapogenins by the same solvent system.

Infra-red analysis—Characteristic bands of pure diosgenin and yamogenin (free from each other) are summarized in Table 3.2. Figure 3.5 illustrates the pertinent peaks for the estimation of the two epimers and their mixtures. The calibration for the sample at 900 cm^{-1} was very similar to that obtained by Brain *et al.* but calibration for yamogenin at 920 cm^{-1}showed lower absorbance values compared to sarsasapogenin at 920 cm^{-1}, both epimer graphs were linear over the range 0.2 to 1.0 g/100 ml.

The D:Y ratio of an unknown mixture (D + Y) can be obtained from the ratio of absorbance values at 920 and 900 cm^{-1}. From data obtained by IR analysis by mixing separated epimers and expressed as ratio graph (Fig. 3.10), D:Y ratio of an unknown sample can be determined. The absorbance ratio is also a useful way of expressing the purity of an epimer. Two methods of calculations were used (1) direct method, where either D or Y was markedly predominant and (2) the ratio method where D and Y were approximately equal in amount. With direct method, total sapogenin concentration was calculated from 980 cm^{-1} graph and concentration of predominant sapogenin from the total sapogenin value. Ratio method needs the diosgenin graph at 900 cm^{-1} prepared in the presence of known amount of yamogenin, a yamogenin graph at 920 cm^{-1} and a ratio graph (Figs. 3.5 and 3.10).

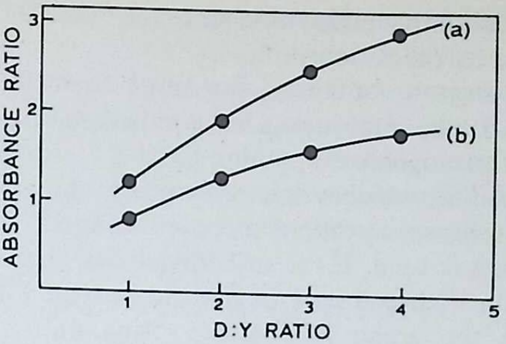


Fig. 3.10—D:Y Ratio graph: Curve (a) is obtained from the absorbance ratio 900 ÷ 920 against D:Y ratio of concentration and is used for mixtures containing more diosgenin than yamogenin; Curve (b) is similarly obtained from 920 ÷ 900 and is used when yamogenin is in excess of diosgenin.

Accuracy of the calibrations—Diosgenin and yamogenin were weighed separately to test the direct method at 0.5% w/v and then together at the same concentration to test the ratio method. This also gave a direct comparison of the values for the individual epimers calculated by both methods. The effect of total sapogenin concentration on the error of the direct and ratio methods was checked by using D:Y ratios 9:1, 1:1 and 1:9, each at four concentrations.

Recommended calculation method—In all unknown mixtures the ratio of absorbance at 920 and 900 cm^{-1} will indicate the D:Y concentration ratio and hence the method of calculation. The methods recommended by Jefferies & Hardman (36a) are reproduced here *verbatim.*

I. For diosgenin containing not more than 20% yamogenin, use the direct method. The total sapogenin is calculated from the 980 cm^{-1} absorbance using the 994 cm^{-1} baseline or 984 cm^{-1} baseline if present. The yamogenin content is the difference between the total sapogenin content and the diosgenin content. If the mixture contains up to 10% yamogenin the diosgenin content is calculated from the 900 cm^{-1} graph prepared with pure diosgenin. If the mixture contains up to 20% yamogenin then the 900 cm^{-1} graph should be prepared in the presence of 20% of yamogenin. At 20% yamogenin, the ratio method could also be used.

II. For diosgenin containing 20 to 50% yamogenin, use the ratio method. The total sapogenin is the sum of the individual sapogenin values. The diosgenin content is obtained from a diosgenin 900 cm^{-1} graph prepared in the presence of yamogenin, as indicated by the D:Y concentration ratio. Separate graphs (Fig. 3.10) are needed for D:Y ratios of 1:1 and 2:1 to 4:1. The yamogenin content is calculated from the diosgenin value by simple proportion since the exact D:Y concentration ratio is provided by the ratio graph.

III. For yamogenin containing not more than 25% diosgenin use the direct method. The total sapogenin is calculated from the 980 cm^{-1} absorbance. For yamogenin containing 10 to 25% diosgenin use the 996 or 990 cm^{-1} baseline, whichever is present. For yamogenin containing less than 10% diosgenin, a graph is prepared using 5% diosgenin and the 990 cm^{-1} baseline is used. If the instrument has an automatic marker device at 1000 cm^{-1} then it may be used to provide a false shoulder at 996 cm^{-1} when the graph is obtained using pure yamogenin. The yamogenin content is obtained from the 920 cm^{-1} graph for pure yamogenin. The diosgenin content is given by the difference between total sapogenin and yamogenin value.

IV. For yamogenin containing 25 to 50% diosgenin, use the ratio method. The total sapogenin is the sum of the individual sapogenin values. The yamogenin content is obtained from a 920 cm^{-1} graph for pure yamogenin. The diosgenin content is calculated from the yamogenin value by simple proportion since the exact D:Y concentration ratio is provided by the ratio graph.

The methods recommended above enable diosgenin and yamogenin to be estimated separately and together in all proportions. In practice for a given species and morphological part, the D:Y ratio is in a narrow range, and calibrations need only be made in this range, e.g. fenugreek seeds usually have a D:Y ratio of the order of 6:4. Another simplification for the ratio method calibrations is to use 1% w/v solutions for each D:Y ratio required; the solutions may then be progressively diluted and assayed. The results will provide graphs for the major sapogenin in the presence of the minor sapogenin as well as the ratio graph.

Range of error—Over the concentration range 1.0-0.50% w/v, the range of error by Studentized ($p = 0.99$) Range Test was found to be for the direct method 1.4-3.7% for total sapogenin and 2.1-4.3% for predominant epimers, and for the ratio method 1.8-3.1 for total sapogenin and 2.2-6.3% for individual epimers. When the minor epimer is only 10% of the sapogenin mixture, its concentration is too low for its accurate estimation by IR analysis.

Column Chromatography—IR Spectrophotometry

For the study of *Trigoenella foenum-graecum* seed as a commercial source of diosgenin and yamogenin, it is important that both the epimers be accurately determined; the yamogenin usually accounts for 40 per cent of the mixture of the two sapogenins. A breeding programme for seed, with high sapogenin, protein and fixed oil contents, requires a method suitable for a large number of determinations. Hardman & Jefferies (36c) developed a routine procedure involving the use of silica gel column in separating diosgenin and yamogenin. They (36b) further improved the column chromatographic technique by using water-containing solvents. The advantages are that there is less variation between duplicate results, that each column can be used at least five times and that component bands are eluted in predictable volumes of solvents. The apparatus and solvent sequence described here allow 12 columns to be developed simultaneously.

Materials—Crude extracts obtained by acid hydrolysis method.

Design of the column apparatus—Twelve Whatman precision chromatographic columns (20 × 1 cm) were mounted close together on a vertical board. Solvent-proof connections between PTFE tubing and glass tubing were made by making PTFE connectors of precise dimensions, the outside diameter of the connector was slightly larger than the internal diameter of the glass tubing and the internal diameter of the central bore of the connector was slightly less than the outside diameter of the PTFE tubing. Above each column, a 3-way tap was fitted which led to a specimen tube and to a solvent reservoir of 2-litre capacity. The specimen tube was used to siphon the plant crude extract into the column. The outlet of the solvent reservoir was a PTFE stopper carrying twelve PTFE tubes, one to each column. Below each column was a PTFE-tube S-band with a tap fitted in the middle of the S-band which prevented air in the tap from reaching the adsorbent in the column.

Column procedure—Water saturated solvent mixtures of hexane and ethyl acetate in proportions of (9 + 1), (3 + 1) and (2 + 3) were used. Silica-gel for adsorption, activity II, 6 g per Whatman Column (20 × 1 cm), was packed in hexane-ethyl acetate (9 + 1). Crude sapogenin extracts were prepared and added to the columns.

At a flow-rate of 1 ml/min, the sequence of hexane-ethyl acetate mixtures (used for the elutions and the fractions collected) was: 9 + 1 mixture—40 ml containing fixed oil, steryl esters and spirostadienes, then 45 ml containing free sterols, followed by the three 5-ml fractions of a thin-layer chromatographic check; 3 + 1 mixture—40 ml containing diosgenin and yamogenin (plus tigogenin and nectigogenin when present), followed by three 5-ml fractions for a thin-layer chromatographic check; and 2 + 3 mixture—20 ml containing trace compounds, then 30 ml containing dihydroxysapogenins (such as gitogenin). Finally, ethyl acetate was used and a 25-ml fraction collected that contained column residues. For the regeneration of the column, 10, 10 and 25 ml of the 2 + 3, 3 + 1 and 9 + 1 mixtures respectively, were used; the column could then be re-used.

The method has been found to be satisfactory for column loadings of up to 75 mg of D + Y in the presence of up to 600 mg of fixed oil which is approximately three times the amount of the oil present in 2.5 g of sample of Moroccan fenugreek seed.

For estimation see Recommended calculation method under *P*-TLC—IR Spectrophotometric procedure.

It has now become possible to separate the 4 sapogenins, namely D, Y, tigogenin & neotigogenin by the use of argentation TLC (37). The sapogenin extracts purified by column chromatography were examined by this method and the results assessed visually against a range of standard solutions. (Densitometric scanning of the plates with a Joyce-Loebl Chromoscan was unsuccessful because of the colour of the spots). In fenugreek seed, about 10% of the D + Y content was found to be due to tigogenin and neotigogenin, each contributing about 5% to the spectrophotometric result.

The suitability of the method for the analysis of other plant extracts that had different contents of sterol, D and fixed oil was tested on crude extracts of *D. deltoidea* tuber, which afforded a non-oily sapogenin extract, and fruits of *B. aegyptiaca*, a material with seed containing up to 50% of fixed oil. The sequence of solvents used and the fractions collected for fenugreek seed were found to be satisfactory for these plant samples. The tubers of *D. deltoidea* and the fruit wall and seed of *B. aegyptiaca* did not show any tigogenin or neotigogenin.

The error introduced into the IR spectrophotometric assay by omitting the column procedure and analysing the crude extract was directly studied. The D + Y percentages with D:Y ratios are presented in Table 3.4 for non-oily and oily plant materials. The data indicate that in the presence of fixed oil component, not only is the isolated D + Y value

TABLE 3.4—COMPARISON OF SAPOGENIN CONCENTRATIONS AND D:Y RATIOS BY IR ANALYSIS OF CRUDE AND PURIFIED EXTRACTS FROM NON-OILY AND OILY PLANT PARTS[1]

	IR analysis of crude extract		Column chromatography—IR analysis	
	D + Y %	D:Y ratio	D + Y %	D:Y ratio
D. deltoidea rhizome	5.3	99:1	4.8	99:1
B. aegyptiaca fruit wall	1.2	48:52	1.1	48:52
B. aegyptiaca seed (oily)	2.0	74:26	1.0	92:8
T. foenum-graecum seed (oily)	1.5	50:50	1.0	58:42

[1] Jafferies & Hardman, *Analyst*, 1976, **101**, 122-24.

TABLE 3.5—THE RELATIVE MERITS AND DEMERITS OF VARIOUS ASSAY PROCEDURES FOR DIOSGENIN

Merits	Demerits	Remarks
	1. GRAVIMETRIC	
Easy; no sophisticated instrument necessary, except the Meltars balance	Approximate estimation; requires at least 50g of plant material; the whole of the extract or at least most of it should be sapogenin	Suitable for the estimation of diosgenin from *Dioscorea*, *Costus*, *Kallstroemia*, etc.; used by commercial extractors
	2. COLORIMETRIC	
Only 50mg of the dried plant material required; rapid and accurate, 8 to 10 samples per day can be assayed; no significant interference from phytosterol and limited amounts of fixed oil	Interference from other sapogenins such as pennogenin; 25*R*- & 25*S*- epimers not estimated separately	Suitable for the estimation of diosgenin from *Dioscorea* or samples from plant survey or breeding programme
	3. GLC	
About 5g of undried plant material adequate; dienes, sterols can be separated	25*R*- and 25*S*- epimers not separated	Suitable for the estimation of diosgenin from *Dioscoreas* in a breeding programme or in tissue culture
	4. DENSITOMETRIC TLC	
Small amount of plant material adequate; rapid and accurate; can separate diosgenin from dienes and sterols	The pair of 25*R*- & 25*S*- sapogenins not easily separable; preparative TLC necessary to separate the epimers	Suitable for the estimation of diosgenin from *Dioscoreas* in a breeding programme or in tissue culture
	5. IR SPECTROPHOTOMETRY	
1 to 10g of undried material sufficient; rapid, 100 to 150 samples per week (40 hours); 25*R*- and 25*S*- epimers estimated	Cannot estimate 3:5 dienes (of both 25*R*- & 25*S*-); interference due to absorption by sterols and fixed oil; yamogenin content highly exaggerated; sample must yield minimum of 50mg of sapogenin	Appropriate for the study of the variation in C-25 epimers in morphological parts and with season for routine screening of steroid sapogenins
	6. *P*-TLC—IR SPECTROPHOTOMETRY	
2.5g of sample sufficient; can estimate D & Y separately and together in almost all proportions; eliminates most of the impurities	If the minor epimer, (generally Y) is only 10% of the sapogenin mixture the amount is too low to be estimated accurately	Suitable for the estimation of diosgenin/yamogenin individually and totally with accuracy from fenugreek and *Balanites* seeds
	7. COLUMN CHROMATOGRAPHY—IR SPECTROPHOTOMETRY	
Same as *P*-TLC—IR analysis. Simultaneous isolation of sterols and gitogenin. Column can be used at least 5 times	Does not permit the separation of diosgenin from tigogenin, and yamogenin from neotigogenin; D + Y content also includes the said 5α & β sapogenins	The only suitable method for the estimation of D/Y individually and totally with near accuracy from fenugreek seed in a breeding programme. The method can be applied to *Balanites* seeds as well

greatly exaggerated, but the properties calculated as D or Y are incorrect. These effects are due to greater interference taking place at 920 cm^{-1}, which increases the apparent Y value, as previously reported.

The improved method could also be applied to the determination of individual amounts of other pairs of (25 *R*)- and (25 *S*)-spirostan-3β-ols, which may be present in any proportion, for example, smilagenin and sarsasapogenin, and gitogenin [(25 *R*)-5α-spirostan-2α,3β-diol] and neotigogenin. The method does not enable the individual amounts of monohydroxy (25 *R*)-spirostan-3β-ols (e.g. D, tigogenin and smilagenin) to be determined in a mixture of these in the presence of their (25 *S*)-epimers.

REFERENCES

1. Fieser, L.F. & Fieser, M. Steroids (Reinhold Publishing Corporation, New York), 1959, 811.

2a. Revised tentative rules for nomenclature of steroids, *in* Rodd's Chemistry of Carbon Compounds, edited by Coffey, S. (Elsevier Publishing Co., Amsterdam, London, New York), 2nd ed., Vol. II, Part D, 1970, 432-34; 2b. Elks, J. Steroid saponins and sapogenins, *in* Vol II, Part E, 1971, 25.

3. Das Gupta, B. & Pandey, V.B. A new Indian source of diosgenin (*Costus speciosus*). *Experientia*, 1970, **26,** 475-76.

4. Chakravarti, R.N., Mahato, S.B., Sahu, N.P., Pal, B.C. & Chakravarti, D. *Kallstroemia pubescens*—a new source for diosgenin. Part I. *J. Inst. Chem. India*, 1976, **48,** 170-73.

5. Hardman, R. Pharmaceutical products from plant steroids. *Trop. Sci.*, 1969, **11,** 196-228.

6a. Dictionary of Organic Compounds, edited by Heilbron, I. and others (Eyre & Spottiswoode Publishers Ltd., London), 4th ed., Vol. 3, 1965, 1545, 1262; 6b Vol. 5, 1965, 3154.

7. Ghosal, S., Srivastava, R.S., Chatterjee, D.C. & Datta, S.K. Fenugreekine, a new steroidal ester of *Trigonella foenum-graecum. Phytochemistry*, 1974, **13**, 2247-51.

8. Bedi, K.L., Sarin, Y.K. & Atal, C.K. *Costus speciosus* as commercial source of diosgenin. *Indian J. Pharm.*, 1976, **38,** 155-56.

9a. Information from Dr. Y.K. Hamied, Managing Director, CIPLA, Bombay; 9b. Information through personal communication or discussion.

10. Rajaraman, K., Seshadri, V. & Rangaswami, S., Prazerigenin-A-----glycosides, two new glycosides from *Dioscorea prazeri* and a new saponin from *D. deltoidea. Indian J. Chem.*, 1976, **14B,** 735-38.

11. Rajaraman, K., Batta, A.K. & Rangaswami, S. Structures of three new steroidal sapogenins from *Dioscorea prazeri. J. Chem. Soc., Perkin Trans. I.*, 1975, 1560-62.

12. Wij, M., Rajaraman, K. & Rangaswami, S. New glycosides of diosgenin and Prazerigenin-A from the rhizomes of *Dioscorea prazeri. Indian J. Chem.*, 1977, **15B,** 451-54.

13. Sofowora, E.A. & Hardman, R. Chromatographic detection and spectrophotometric determination of diosgenin and other Δ^5-sapogenins in crude plant extracts. *Planta Med.*, 1974, **26,** 385-90.

14. Fazli, F.R.Y. & Hardman, R. The spice, fenugreek (*Trigonella foenum-graecum* L.). Its commercial varieties of seed as a source of diosgenin. *Trop. Sci.*, 1968, **10,** 66-78.

15. Dawider, A.A.M. & Fayez, M.B.E. Steroid sapogenins—XIII. The constituents of *Balanites aegyptiaca. Phytochemistry*, 1969, **8**, 261-65.

16. Hardman, R. & Wood, C.N. The ripe furits of *Balanites orbicularis* as a new source of diosgenin and yamogenin. *Phytochemistry*, 1971, **10**, 887-89.

17. Hardman, R. & Wood, C.N. The effect of ripening and aqueous incubations on the yield of diosgenin and yamogenin from the fruits of *Balanites pedicellaris. Planta Med.*, 1971, **20**, 350-56.

18. Applezweig, N., Steroid Drugs (McGraw-Hill Book Co., Inc., New York), 1962, 46-47.

19. Brain, K.R., Fazli, F.R.Y., Hardman, R. & Wood, A.B. The rapid quantitative determination of C-25 epimeric steroidal sapogenins in plants. *Phytochemistry*, 1968, **7**, 1815-23.

20. Peal, J.W. Dehydration of diosgenin during the acid hydrolysis of *Dioscorea* saponins. *Chem. & Ind.*, 1957, 1451-52.

21. Wall, M.E., Eddy, C.R., McClennan, M.L. & Klumpp, M.E. Detection and estimation of steroidal sapogenins in plant tissue. *Anal. Chem.*, 1952, **24**, 1337-41.

22. Yamauchi, T. Saponins of Japanese *Dioscoreaceae* IX. Hydrolysis of diosgenin glycosides. *Chem. Pharm. Bull.*, 1959, **7**, 343-48 (through *Chem. Abstr.*, 1960, **54**, 24855-56).

23. Shoppee, C.W., Chemistry of the Steroids (Butterworths, London), 2nd ed., 1964, 403.

24a. Chakravarti, R.N., Chakravarti, D. & Mitra, M.N. By-products in the isolation of diosgenin from Indian *Dioscorea* yams. *J. & Proc. Inst. Chem. India*, 1960, **32**, 22-25; 24b. Isolation and reactions of steroid sapogenins. *J. Indian Chem. Soc.*, 1961, **38**, 635-41.

25. Rothrock, J.W., Hammes, P.A. & McAleer, W.J. Isolation of diosgenin by acid hydrolysis of saponin. *Ind. Eng. Chem.*, 1957, **49**, 186-88.

26. Blunden, G., Hardman, R. & Morrison, J.C. Quantitative estimation of diosgenin in *Dioscorea* tubers by densitometric thin-layer chromatography. *J. Pharm. Sci.*, 1967, **56**, 948-50 (through *Chem. Abstr.*, 1967, **67**, 84897).

27. Hardman, R., Wood, C.N. & Brain, K.R. The resolution of 25α- and 25β-spirosta-3,5-dienes from sapogenin yielding plants. *Phytochemistry*, 1972, **11**, 2073-76.

28. Paul, V., Handa, K.L. & Chopra, I.C. Diosgenin. *Chem. Abstr.*, 1960, **54**, 7983.

29. U.S. Patent No. **3,505,316**, 1970 (through *Chem. Abstr.*, 1970, **73**, 28903).

30. Hardman, R. & Brain, K.R. The effect of post harvest application of growth regulators on the yield of steroidal sapogenins from plant material. *Phytochemistry*, 1971, **10**, 519-23.

31. Information from Shri B.C. Gulati, Scientist-in-Charge, Drug Research Laboratory (CIMPO), Jammu-Tawi.

32. Selvaraj, Y., Subhas, C.M., Bammi, R.K. & Randhawa, G.S. Preliminary note on the effect of incubation of fresh tubers of dioscoreas on diosgenin content. *Curr. Sci.*, 1972, **41**, 499-500.

33a. Hardman, R. & Sofowora, E.A. A reinvestigation of *Balanites aegyptiaca* as a source of steroidal sapogenins. *Econ. Bot.*, 1972, **26**, 169-73; 33b. Isolation and characterization of yamogenin from *Balanites aegyptiaca*, *Phytochemistry*, 1970, **9**, 645-49.

34a. Khanna, P. & Mohan, S. Isolation & Identification of diosgenin and sterols from fruits and *in vitro* cultures of *Momordica charantia* L. *Indian J. Exp. Biol.*, 1973, **11**, 58-60; 34b. Khanna, P., Khanna, R., Sogani, M. & Manot, S.K. *Daucus carota* L. seedling callus, a new source of diosgenin. ibid., 1977, **15**, 586-87.

35a. Kaul, B. & Staba, E.J. Dioscorea tissue cultures. I. Biosynthesis and isolation of diosgenin from *Dioscorea deltoidea* callus and suspension cells, *Lloydia*, 1968, **31**, 171-79; 35b. Kaul, B., Stohs, S.J. & Staba, E.J. *Dioscorea* tissue cultures. III.

Influence of various factors on diosgenin production by *Dioscorea deltoidea* callus and suspension cultures. ibid., 1969, **32,** 347-59.

36a. Jefferies, T.M. & Hardman, R. The infra-red spectrometric estimation of diosgenin and yamogenin individually and as their mixtures. *Planta Med.*, 1972, **22,** 78-87; 36b. An improved column chromatographic quantitative isolation of diosgenin and yamogenin from plant crude extracts prior to their determination by infra-red spectrophotometry. *Analyst*, 1976, **101,** 122-24; 36c. Hardman, R. & Jefferies, T.M. A combined column-chromatographic and infra-red spectrophotometric determination of diosgenin and yamogenin in fenugreek seeds. ibid., 1972, **97,** 437-41.

37. Dawidar, A.M., Saleh, A.A. & Elmotei, S.L. Steroid sapogenin constituents of fenugreek seeds. *Planta Med.*, 1973, **24,** 367-70.

38. International Trade Centre UNCTAD/GATT Markets for Selected Medicinal Plants and their Derivatives. Geneva, 1974, Chapter IV—Dioscoreas and Diosgenin, 81.

39. Information from Shri L.C. Rabha, Scientist, RRL, Jorhat (Assam).

40. Morris, M.P., Roark, B.A. & Cancel, B. Simple procedure for the routine assay of *Dioscorea* tubers. *J. Agric. Food Chem.*, 1958, **6,** 856-58.

41. Chakravarti, R.N., Chakravarti, D. & Mitra, M.N. A simplified method for isolation of diosgenin from Indian dioscorea yams. *J. & Proc. Inst. Chem., India*, 1958, **30,** 106-12.

42. Preston, W.H. (Jr), Haun, J.R., Garvin, J.W. & Daum, R.J. Several aspects of growth, development and sapogenin yield of tubers of *Dioscorea spiculiflora*. *Econ. Bot.*, 1964, **18,** 323-28.

43. Chakravarti, R.N., Dash, S.N. & Chakravarti, D. Estimation of diosgenin content of *Dioscorea* yams. *J. Inst. Chem. India*, 1970, **42,** 165-68.

44. Selvaraj, Y. Dioscorea improvement II. Studies on the standardization of diosgenin assay procedure. *Indian J. Hort.*, 1971, **28,** 135-38.

45. Yamagishi, M. & Nakamura, I Determination of sapogenins in *Dioscorea* species from Japan. I. Colorimetric determination of diosgenin and tokorogenin. *Yakugaku Zasshi*, 1958, **78,** 1068-71 (through *Chem. Abstr.*, 1959, **53,** 1636).

46. Rishi, A.K., Kapoor, R. & Vakhlu, V.K. A rapid spectrophotometric method for the quantitative determination of diosgenin in *Dioscorea* tubers. *Curr. Sci.*, 1976, **45,** 327-28.

47. Cooke, B.K. Determination of diosgenin in *Dioscorea deltoidea* and *Dioscorea sylvatica* by using gas-liquid chromatography. *Analyst*, 1970, **95,** 95-97.

48. Dixit, B.S., Srivastava, S.N., Sircar, K.P., Bhatt, G.R. & Tandon, J.S. Estimation of diosgenin in *Dioscorea* tubers by gas-liquid chromatography (Paper presented at the NBG, Lucknow Symposium on Production and utilization of vegetable raw materials for steroid hormones and oral contraceptives, held on March 26-28, 1977).

49. Brain, K.R. & Hardman, R. An improved method of densitometric thin layer chromatography as applied to the determination of sapogenin in *Dioscorea* tubers. *J. Chromatogr.*, 1968, **38,** 355-63.

50. Wall, M.E., Krider, M.M., Krewson, C.F., Eddy, C.R., Willaman, J.J., Correll, D.S. & Gentry, H.S. Steroidal sapogenins. VII. Survey of plants for steroidal sapogenins and other constituents. *J. Am. Pharm. Assoc., Sci. Ed.*, 1954, **43,** 1-7.

51. Chaudhuri, S.B. Standardization of gravimetric method of analysis of *Dioscorea* tubers for diosgenin. *Indian Drugs*, 1977, **14**(6), 128-31.

52. Bennet, R.D. & Heftmann, E. Separation of closely related steroids by an improved technique for continuous development of thin-layer chromatography. *J. Chromatogr.*, 1966, **21,** 488-90.

CHAPTER 4

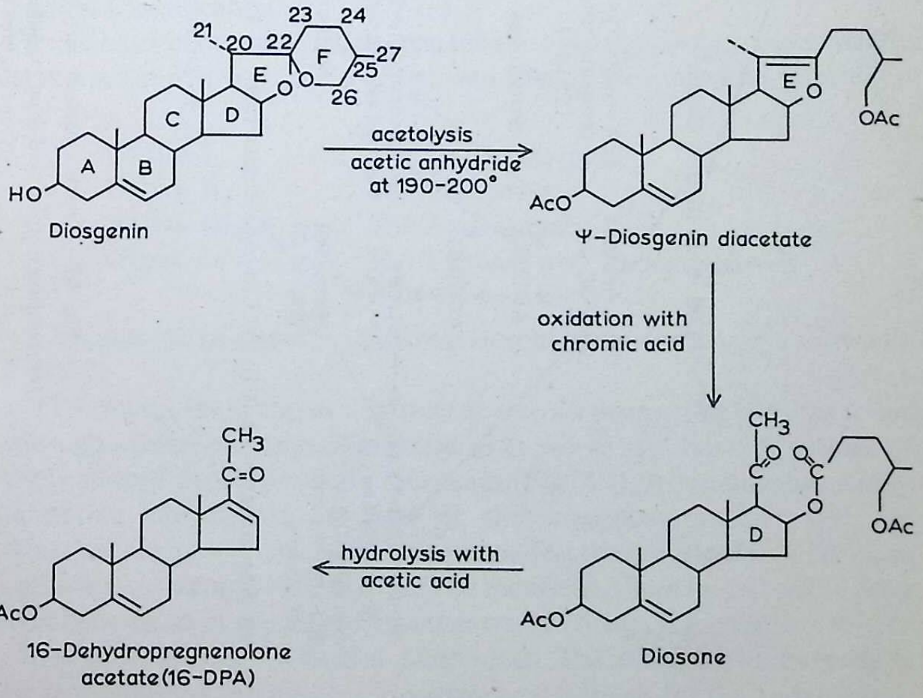
DIOSGENIN CONVERSION TO STEROID DRUGS AND USES

The entire production of diosgenin is first converted to the basic intermediate compound 16-dehydropregnenolone acetate (16-DPA). For conversion to steroid drugs, 16-DPA has to pass through a series of intermediate steps of very complicated nature.

CONVERSIONS

16-DPA

Diosgenin is heated for several hours in a sealed vessel with acetic anhydride at 199 to 200°. This treatment breaks the spiroketal ring F (Fig.4.1) simultaneously introducing 20-22 double bond in ring E. The product formed is pseudodiosgenin (ψ-diosgenin) diacetate, a furostadiene derivative, which on low temperature chromic acid oxidation yields the diketone called diosone in the form of diacetate. This ester on boiling with acetic acid (the present commercial process) or with



21
20
22
23
24
25
26
27
F
E
O
O
C
D
A
B
HO
Diosgenin
acetolysis
acetic anhydride at 190-200°
E
O
OAc
AcO
Ψ-Diosgenin diacetate
oxidation with chromic acid
CH3
C=O
O
C
O
OAc
D
AcO
Diosone
hydrolysis with acetic acid
CH3
C=O
AcO
16-Dehydropregnenolone acetate (16-DPA)

Fig. 4.1—Conversion of diosgenin to 16-DPA.

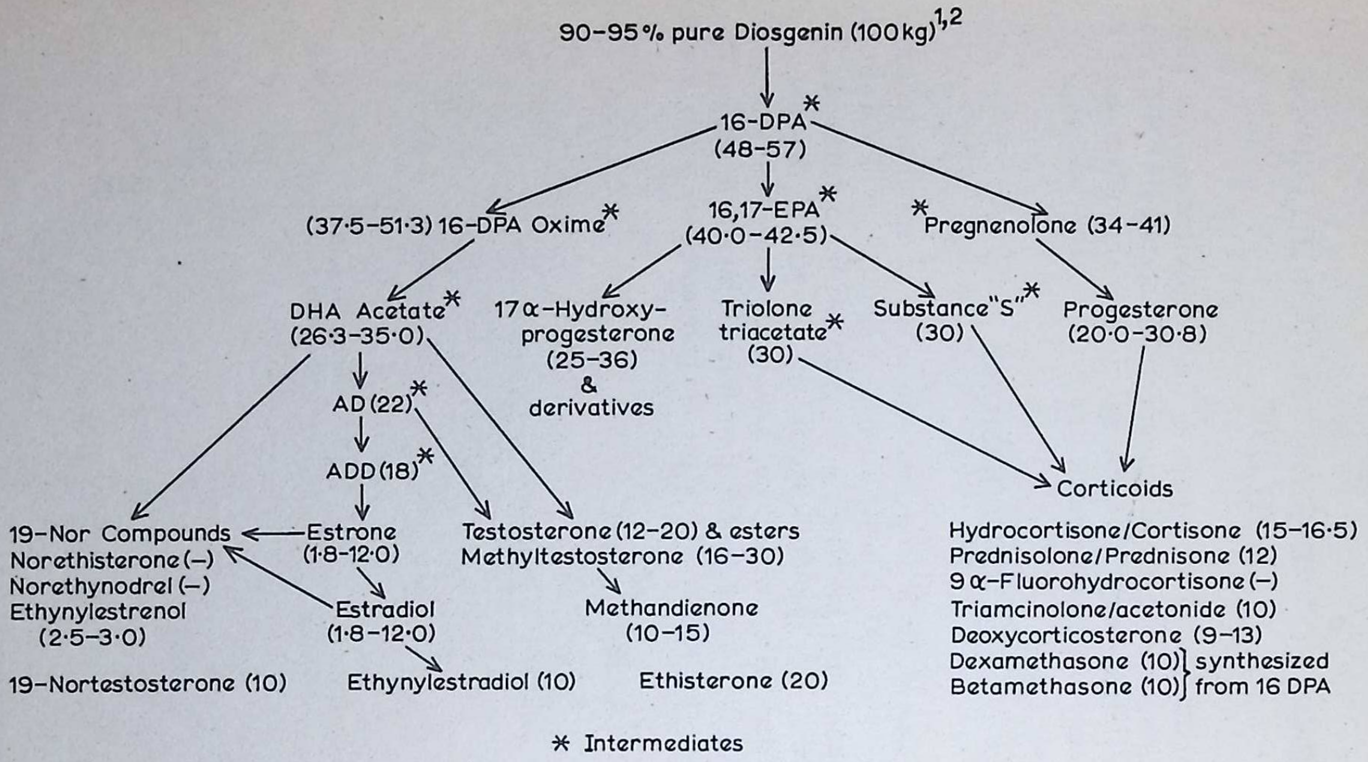

90–95% pure Diosgenin (100 kg)1,2
16-DPA*
(48–57)
(37·5–51·3) 16-DPA Oxime*
16,17-EPA*
(40·0–42·5)
*Pregnenolone (34–41)
DHA Acetate*
(26·3–35·0)
17α-Hydroxy-
progesterone
(25–36)
&
derivatives
Triolone
triacetate*
(30)
Substance "S"*
(30)
Progesterone
(20·0–30·8)
AD (22)*
ADD (18)*
Corticoids
19-Nor Compounds
Norethisterone (–)
Norethynodrel (–)
Ethynylestrenol
(2·5–3·0)
Estrone
(1·8–12·0)
Testosterone (12–20) & esters
Methyltestosterone (16–30)
Hydrocortisone/Cortisone (15–16·5)
Prednisolone/Prednisone (12)
9α-Fluorohydrocortisone (–)
Triamcinolone/acetonide (10)
Deoxycorticosterone (9–13)
Dexamethasone (10)
Betamethasone (10)
synthesized from 16 DPA
Estradiol
(1·8–12·0)
Methandienone
(10–15)
19-Nortestosterone (10)
Ethynylestradiol (10)
Ethisterone (20)
* Intermediates

Fig. 4.2—Product pattern with intermediates and yields from diosgenin.

[1] Information from steroid drug manufacturers; [2] Applezweig, *Chem. Week*, May 17, 1969

(Note: Figures in parentheses are in kg) alkali treatment splits off the remaining side chain readily yielding 16-dehydropregnenolone acetate commonly referred to as 16-DPA (1,2). Although it is a 4-stage reaction (hydrolysis followed by dehydration step is not shown in Fig. 4.1), techniques have been developed to do it by more or less continuous process (3a). Commercially it is of utmost importance to get the maximum yield of 16-DPA.

Several modifications have been suggested as an attempt to obtain better yields; these modifications have been reviewed by Chakravarti *et al.* (4). Use of pyridine hydrochloride as a catalyst in first step is reported to result in more than 80 per cent yield of pseudodiosgenin diacetate (5). In another modification (6), diosgenin was heated with *n*-octanoic acid (b.p. 237°) containing a little acetic anhydride, and low-boiling fractions were distilled off until the temperature reached 240°; it was then refluxed for 2 hours, followed by saponification resulting in free pseudodiosgenin in a yield of 87 per cent. The remaining steps offer no difficulty and 16-dehydropregnenolone can be obtained in yields well above 60 per cent. However, the average industrial yield is seldom so high.

In India, according to some manufacturers of 16-DPA, the yield ranges from 48 to 57 per cent depending on the purity of diosgenin whether it is about 90 or 95 per cent (Fig. 4.2).

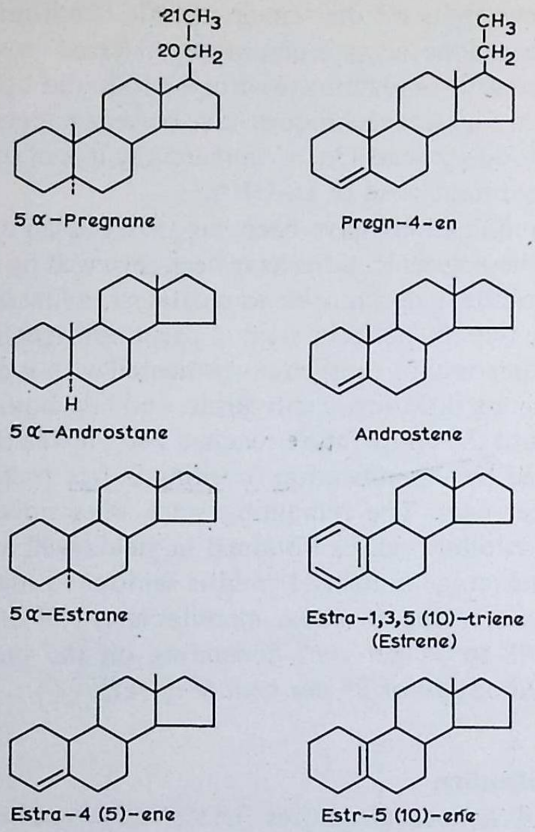
Steroid Classification

On the basis of chemical structures, the steroid drugs, being commercially manufactured in very high purities, can be classified into four groups (7):

Group I	... The Corticosteroids
Group II	... The Pregnenes
Group III	... The Androstenes
Group IV	... The Estrenes and 19-Nor steroids

Various stereo-parents, i.e. parent steroid hydrocarbons, are shown in Fig. 4.3.

To obtain these chemically related steroid drugs, 16-DPA has to go through a series of intermediate steps as shown in molecular flow-sheet of some steroid processes using diosgenin (Fig. 4.4). A manufacturer may terminate the steroid reaction at the intermediate level, sell the intermediate to other manufacturer or convert the same to desired drug as and when the demand arises. It may be mentioned here that a steroid drug may become an intermediate for other steroid drugs, e.g. progesterone for cortisone or estrone for 19-Nor compounds. The following steroids which are not drugs but are pure intermediates or 'midgets' for the above group of compounds, are obtained from 16-DPA:



5 α-Pregnane; Pregn-4-en

5 α-Androstane; Androstene

5 α-Estrane; Estra-1,3,5 (10)-triene (Estrene)

Estra-4 (5)-ene; Estr-5 (10)-ene

Fig. 4.3—Some stereoparents.

Pregnenolone
16,17-Epoxypregnenolone acetate (EPA or 16,17-Epoxide or Epoxy acetate)
Substance "S" or compound "S"
Triolone triacetate
16-Dehydropregnenolone acetate oxime (16-DPA oxime)
Dehydroepiandrosterone acetate (DHA acetate)
Androstenedione (AD)
Androstadienedione (ADD)

All these intermediates except triolone triacetate are produced in India, and are converted to various steroid drugs.

Microbiological Conversions

Steroid conversions are generally very complicated. Most of the intermediates are obtained by purely chemical degradation but AD and ADD as also some drugs like testosterone, methyl testosterone, hydrocortisone and prednisolone are obtained by microbiological conversions or fermentation. In fact, steroid conversions are a combination of chemical reactions and microbiological transformation; the latter reduces a number of chemical steps or operations, is more efficient and gives better yield (3b, 8). Bacteria, filamentous fungi, streptomycetes and even protozoa produce the useful enzymes, but the filamentous fungi are the most versatile (8, 9).

Hydroxylation, dehydrogenation, hydrogenation, epoxidation, and even cleavage of C-17 side chain of the steroid molecule can be achieved by the microorganisms (8). A series of compounds can be obtained by microbiological conversions of substance S and progesterone (Fig. 4.5 and Fig. 4.6).

Corticosteroids

The corticosteroids are derived basically from the hydrocarbon pregnene (Fig. 4.3).

Corticosteroids possess the following functional groups: a keto group at 3 and 20 positions; a double bond between 4 and 5 in Ring A; an oxygen function (keto group or β-hydroxyl group) at position 11 in Ring C (with the exception of substance S and deoxycorticosterone) and a hydroxyl group each on 17-α (deoxycorticosterone, an exception) and 21-carbon atoms. The presence of oxygen functions at positions 3 and 11 and the hydroxyl groups on carbon atoms 17 and 21 seem to be essential for the range of pharmacological activity of these compounds (7).

Of naturally occurring adrenal cortex steroids, only seven are classed as "active"; the group of these steroids may be further subdivided into the highly active glucocorticoids (carbohydrate metabolism), cortisone and hydrocortisone; and into the highly mineralocorticoids (decreasing the reabsorption of potassium and increasing the reabsorption of sodium), deoxycorticosterone and aldosterone. However, glucocorticoids also possess mineralocorticoid activity and vice versa (10, 11, 12a, 13a).

Cortisone, hydrocortisone and deoxycorticosterone are available synthetically for clinical uses as also a number of modifications both in structure and activities particularly of cortisone and hydrocortisone are available. Given below is the list of corticosteroids being manufactured or likely to be manufactured in India:

Hydrocortisone (Cortisol)
Cortisone
Prednisolone
Prednisone
Betamethasone
Dexamethasone
Triamcinolone and its Acetonide

Fludrocortisone acetate
Deoxycorticosterone acetate

The key intermediates used for corticosteroids are substance "S" and triolone triacetate. In India, substance "S" is converted by fermentation to hydrocortisone, which in turn is converted to prednisolone again by fermentation. There are several variations to this sequence and each manufacturer operates his own process best suited to his materials and technology, under the most closely guarded secrecy (7). Fig. 4.4 gives molecular flow-sheet of some steroid processes using diosgenin; included in the Group I is a well known Julian (P.L.) sequence. According to Dr Hamied (3b), in other countries, it is triolone triacetate and not substance "S" that goes for fermentation to make hydrocortisone; a few steps of syntex synthesis from triolone triacetate are included in Fig. 4.4.

Upjohn (U.S.A.) is the only company which uses progesterone for the production of corticosteroids. For this purpose, it obtains its progesterone by fermenting pregnenolone acetate, a common intermediate from diosgenin and stigmasterol.

Betametharone and dexamethasone, having 16β- and 16α-methyl group respectively are 9α-fluorocorticosteroids directly obtained from 16-DPA. Another 9α-fluoro compound, triamcinolone, which has hydroxyl group at 16α-position is synthesized from substance "S". Here again there are variations to the sequence shown in Fig. 4.4. Triamcinolone can also be obtained from hydrocortisone.

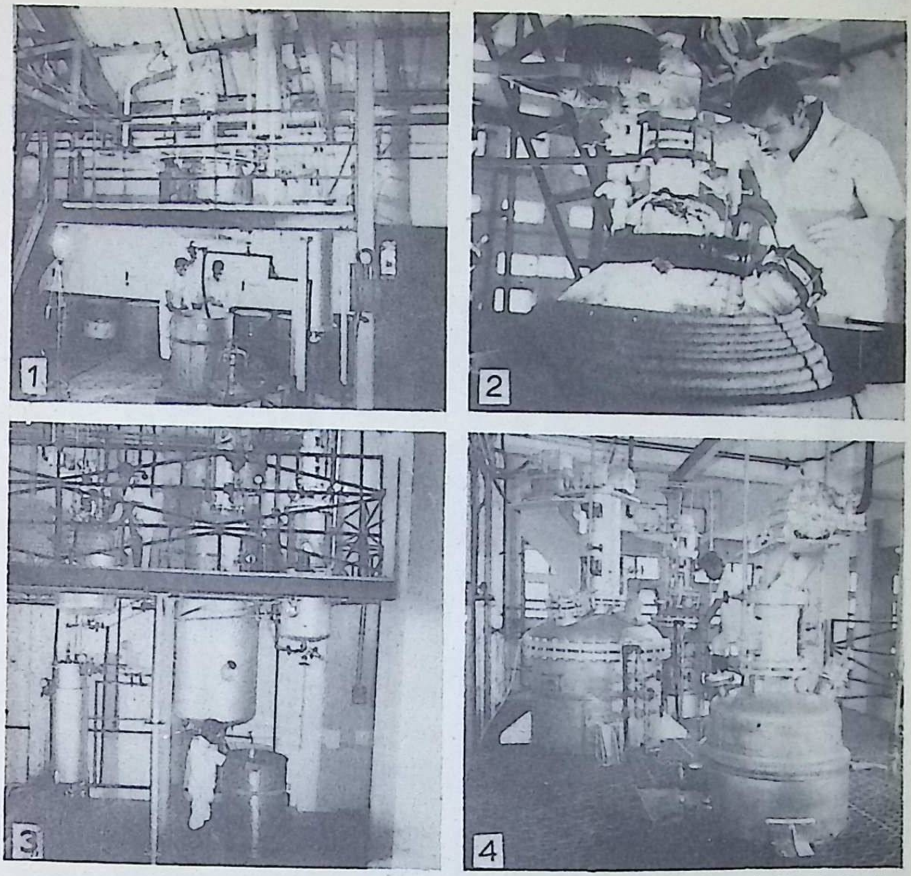
Studies on 'Synthesis and structure-activity relationships in a novel series of topically active corticosteroids' by Green *et al.* (Fifth International Congress on Hormonal Steroids held at New Delhi, on October 29-November 4, 1978) have shown that the 7α-bromo and 7α-chloro derivatives of the 16α-methyl-prednisolone possessed greatly increased topical anti-inflammatory activity over the parent compound. The 17α, 21-dipropionates were more potent than the 21 acetates. The influence of 7α-halogen substitution on topical activity is sensitive to structural changes in remote parts of the molecule. These new steroids can be prepared in good yield by the addition of HBr or HCl to the appropriate 1, 4, 6-trien-3-one. The 7α-halogeno steroids are surprisingly stable, crystalline compounds.

Deoxycorticosterone acetate can be obtained by converting progesterone to 21-iodoprogesterone and subjecting the iodo-derivative to acetoxylation (1).

Yields of some of the corticoids from diosgenin are given in Fig. 4.2.

Pregnenes

This group of compounds all of which have hormonal activities similar to the female hormone, progesterone, are derived from pregnane nucleus

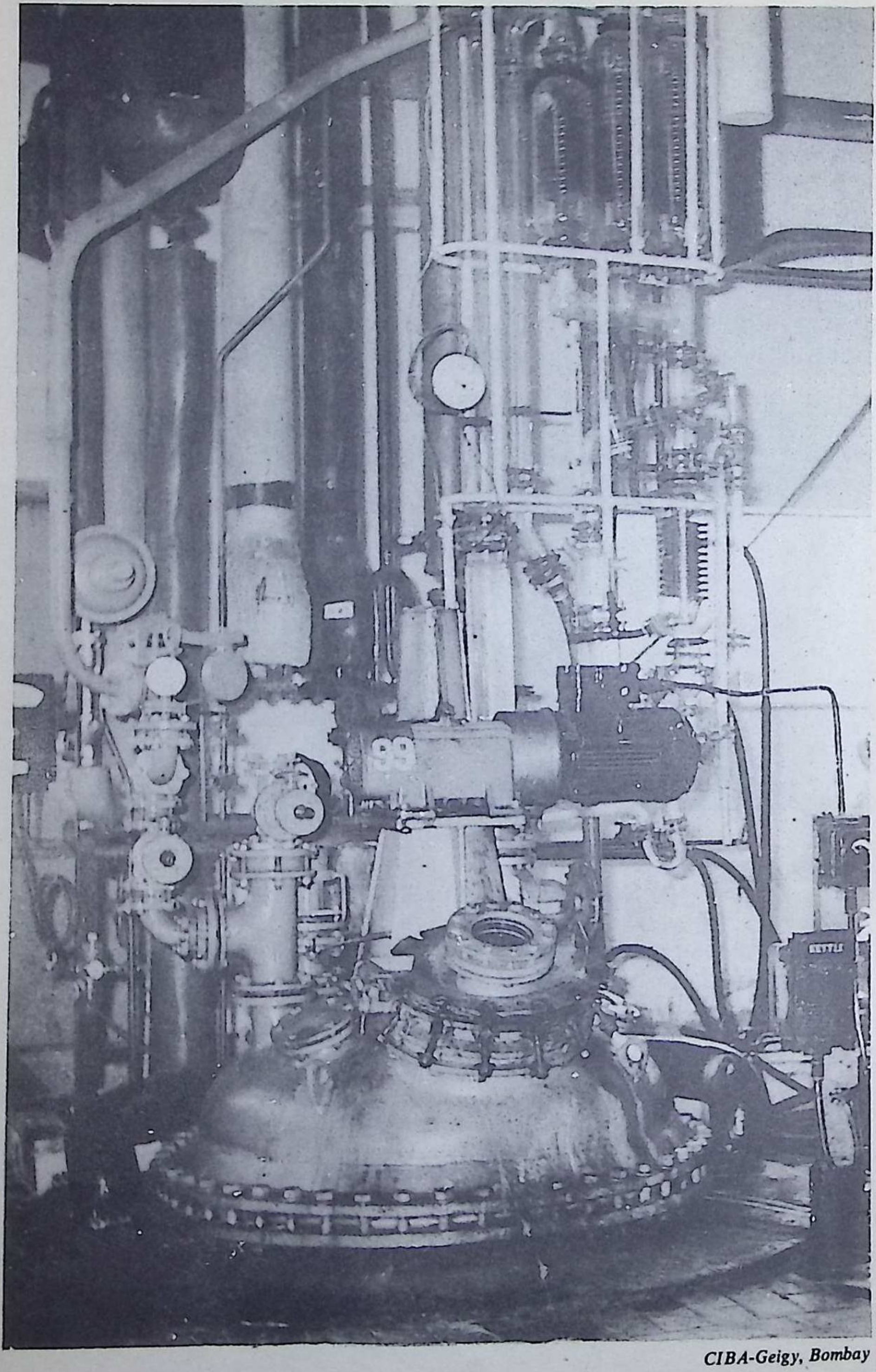


Dr. Y.K. Hamied, CIPLA, Bombay

STEROID CONVERSION PLANTS

1, Reaction view for conducting Oppenauer oxidation; 2, Batet processing of steroid intermediates in Q.V.F. 200 all glass assemblies; 3 & 4, Steroid fermentation units.

Pl IV



CIBA-Geigy, Bombay

MULTIPURPOSE STEROID PLANT

(Fig. 4.3). These compounds possess oxygen functions (keto groups) at 3 and 20 positions and a double bond at 4 and 5 positions like the corticoids but they differ from corticosteroids in that they do not possess any functional group at position 11 and 21 (7).

The compounds under the pregnenes, other than corticoids, include:

Progesterone
17α-Hydroxy progesterone
17α-Acetoxy progesterone & its analogues
17α-Caproyloxy progesterone

Intermediate for these compounds is basically 16-DPA, but progesterone is obtained through pregnenolone and 17α-hydroxy derivatives are obtained through 16, 17-epoxide of 16-DPA (7, 12b).

Androstenes

A series of compounds related to the basic steroid nucleus, androstane (Fig. 4.3) have been obtained by removing the side chain in the pregnane nucleus. Dehydroepiandrosterone acetate (DHA acetate) is the key intermediate for the synthesis of this group of steroids belonging to androstenes (7). They include the following male hormone and its esters (13b) and modifications:

Testosterone and its esters
Methyl testosterone
Methandienone
Ethynyl testosterone

Most of the androgens and anabolics belong to this group.

Spironolactones (14) are synthesized from DHA acetate and chemically they fall in this group; they can be synthesized from AD as well. They act as diuretics and their utilization is dealt with under corticosteroids (Table 4.1).

Estrenes and 19 Nor steroids

A series of compounds related to the estrenes, parent saturated hydrocarbon of which is 5α-estrane (Fig. 4.3), have been obtained by removing the 19-methyl group (No R or Nor) attached to the 10th position in androstene derivatives.

It may be stated here that 19-Nor progesterone and 19-Nor corticoids have not found any importance clinically.

Compounds included under this group are:

Estrone
Estradiol
Ethinylestradiol
19-Nortestosterone
19-Norethisterone (Norethindrone)

(*Contd on page* 106)

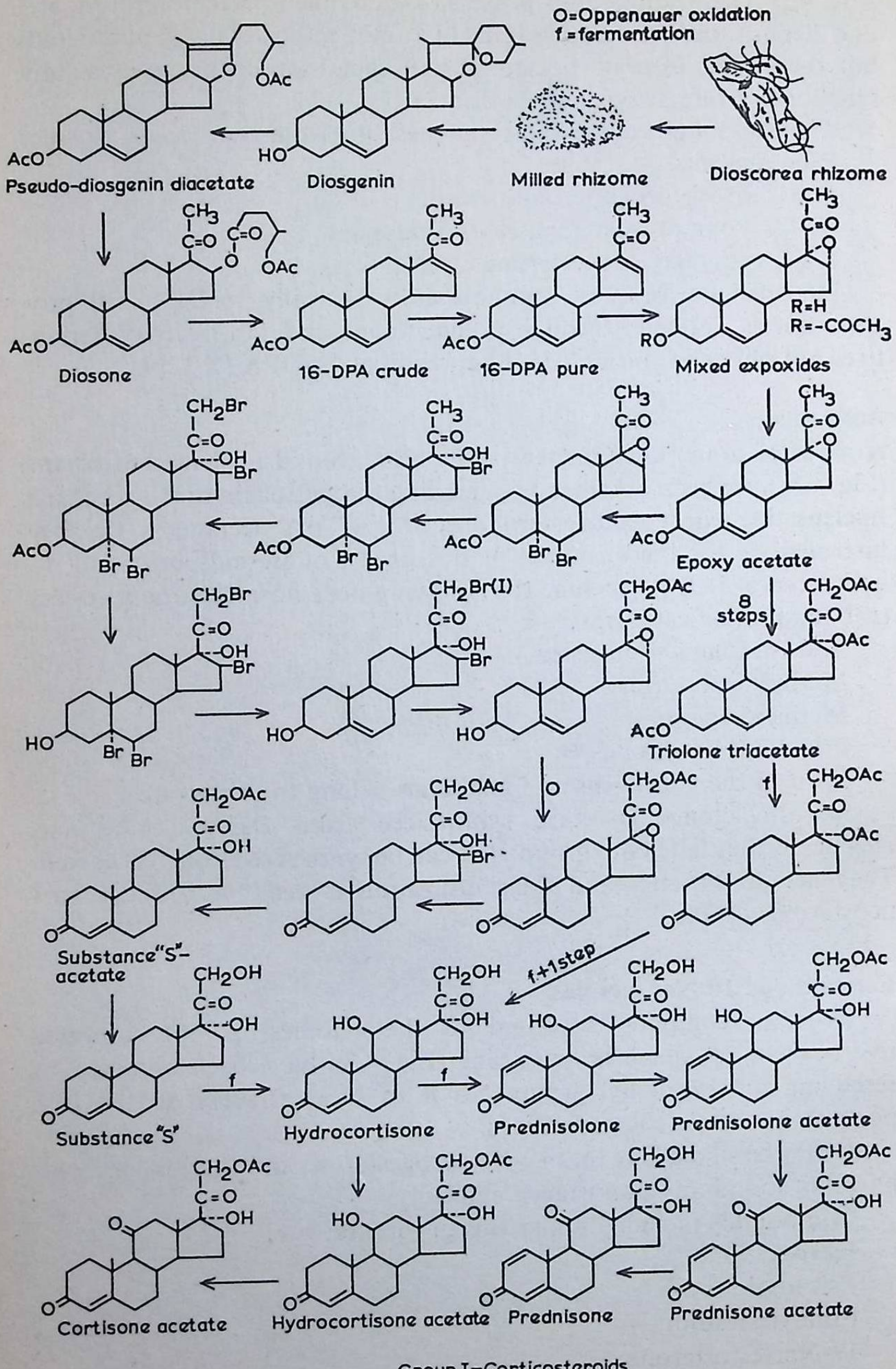
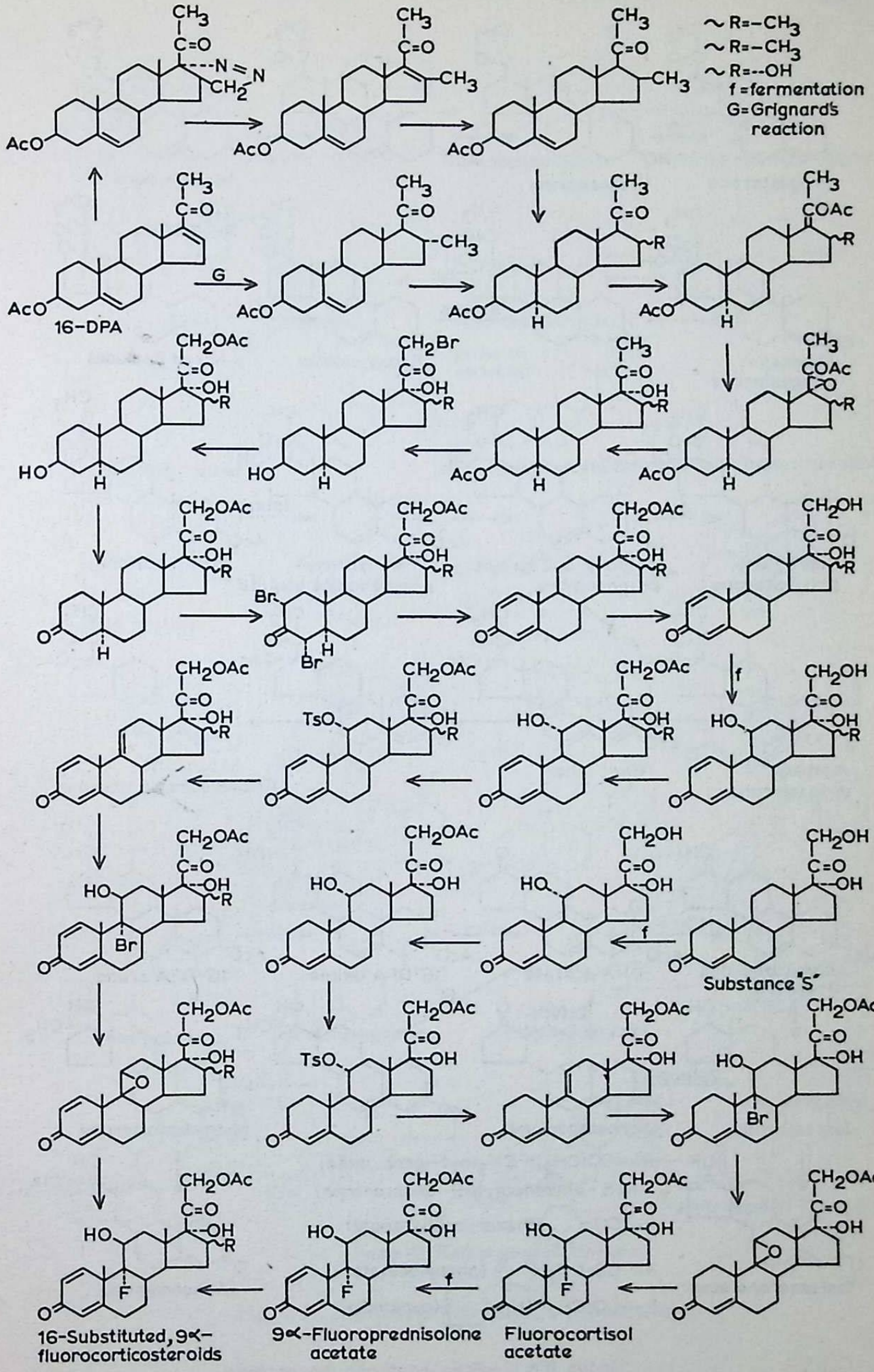
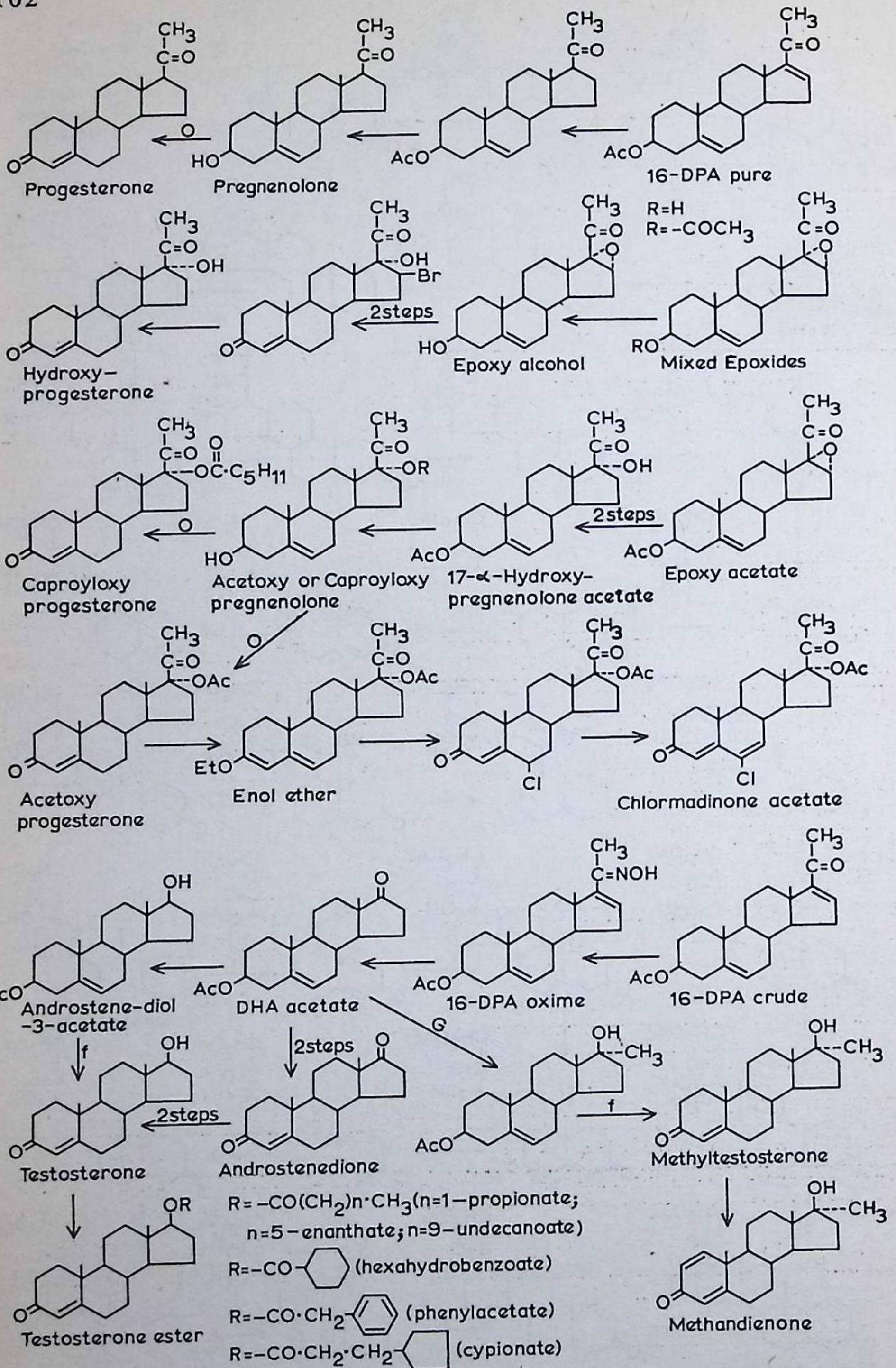

O=Oppenauer oxidation
f=fermentation
Dioscorea rhizome
Milled rhizome
Diosgenin
Pseudo-diosgenin diacetate
Diosone
16-DPA crude
16-DPA pure
Mixed expoxides
R=H
R=-COCH$_3$
Epoxy acetate
8 steps
Triolone triacetate
O
f
f+1step
Substance"S"-acetate
Substance"S"
f
Hydrocortisone
f
Prednisolone
Prednisolone acetate
Cortisone acetate
Hydrocortisone acetate
Prednisone
Prednisone acetate
Group I–Corticosteroids

Fig. 4.4—Molecular flow-sheet of some steroid processes using diosgenin.


~R=–CH3
~R=–CH3
~R=--OH
f = fermentation
G = Grignard's reaction
16-DPA
G
f
Substance "S"
16-Substituted, 9α-fluorocorticosteroids
9α-Fluoroprednisolone acetate
Fluorocortisol acetate

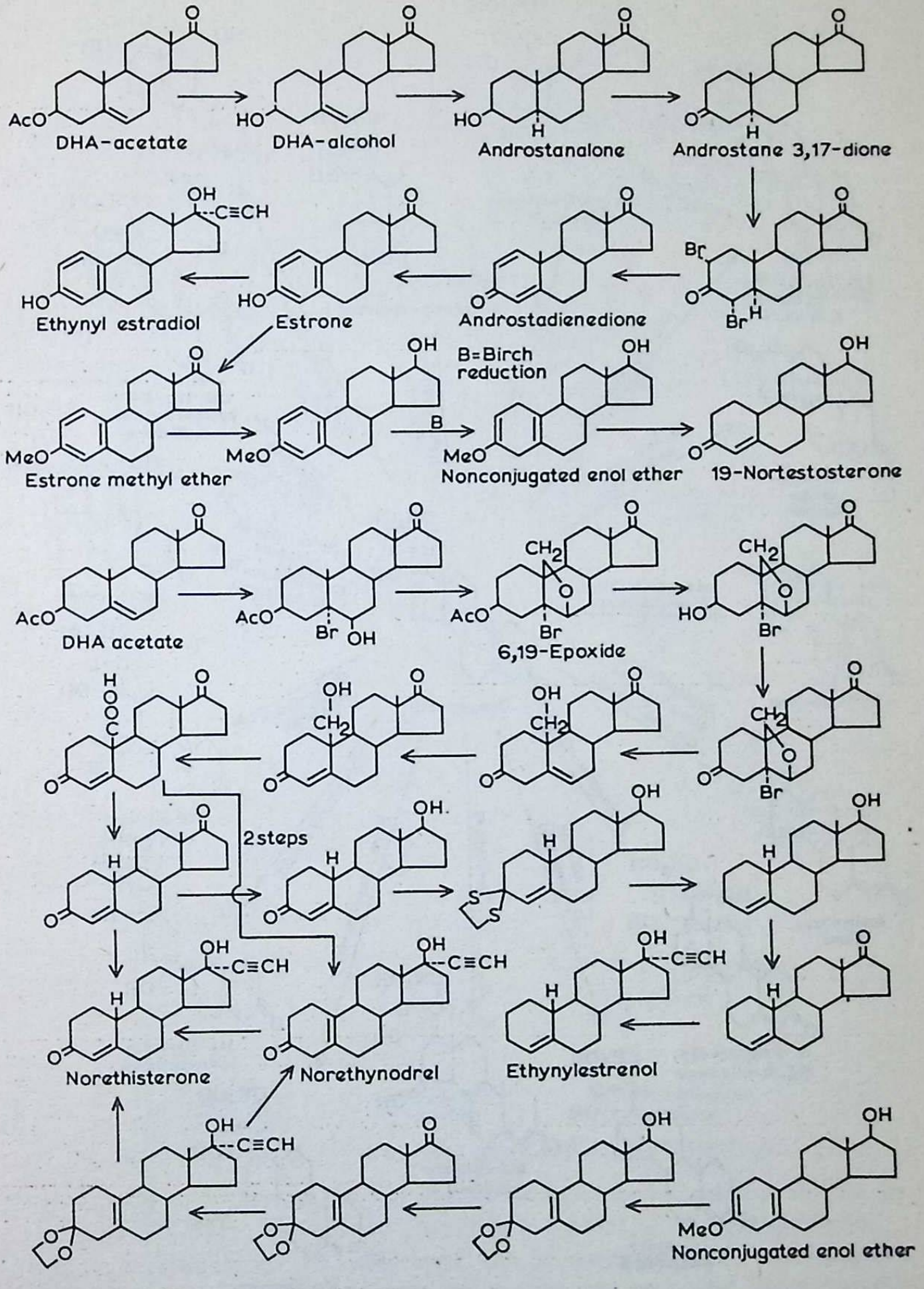
Group I–9α–Fluorocorticosteroids

Fig. 4.4—*Contd.*


Progesterone
Pregnenolone
16-DPA pure
R=H
R=-COCH3
Hydroxy-progesterone
2steps
Epoxy alcohol
Mixed Epoxides
Caproyloxy progesterone
Acetoxy or Caproyloxy pregnenolone
17-α-Hydroxy-pregnenolone acetate
2steps
Epoxy acetate
Acetoxy progesterone
Enol ether
Chlormadinone acetate
Androstene-diol -3-acetate
DHA acetate
16-DPA oxime
16-DPA crude
f
2steps
G
Testosterone
2steps
Androstenedione
f
Methyltestosterone
Testosterone ester
R=-CO(CH2)n·CH3(n=1-propionate; n=5-enanthate; n=9-undecanoate)
R=-CO (hexahydrobenzoate)
R=-CO·CH2 (phenylacetate)
R=-CO·CH2·CH2 (cypionate)
Methandienone

Group II & III—Pregnenes and Androstenes

Fig. 4.4—Molecular flow-sheet—*Contd.*


DHA-acetate
DHA-alcohol
Androstanalone
Androstane 3,17-dione
Ethynyl estradiol
Estrone
Androstadienedione
Estrone methyl ether
B=Birch reduction
B
Nonconjugated enol ether
19-Nortestosterone
DHA acetate
6,19-Epoxide
2steps
Norethisterone
Norethynodrel
Ethynylestrenol
Nonconjugated enol ether

Group IV–Estrenes and 19-Nors

Fig. 4.4—Molecular flow-sheet—(*Concluded*).

[Note—Indian steroid drug manufacturers use 6, 19-epoxide formation to obtain Nor-progestogens]

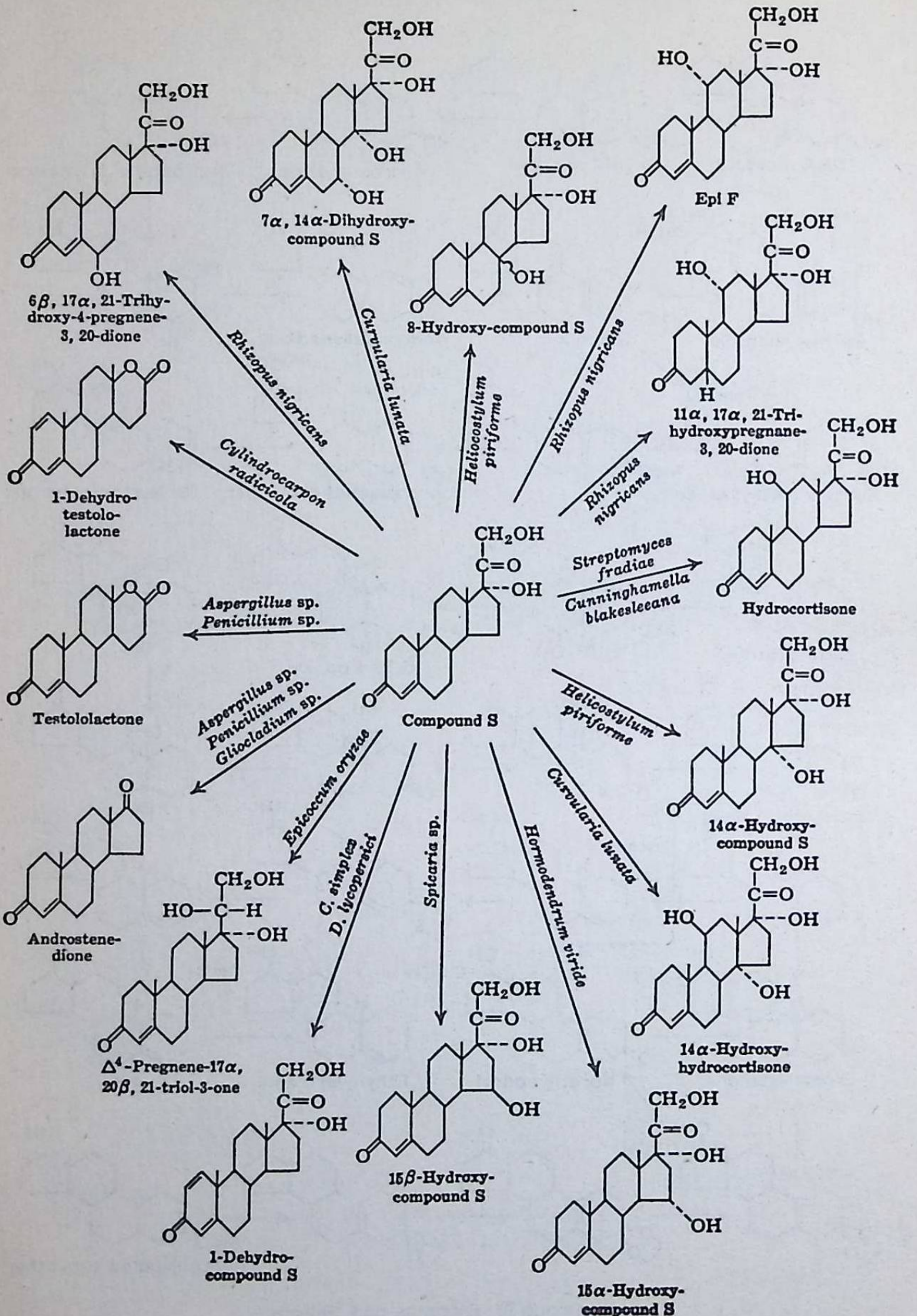

6β, 17α, 21-Trihydroxy-4-pregnene-3, 20-dione
7α, 14α-Dihydroxy-compound S
8-Hydroxy-compound S
Epi F
11α, 17α, 21-Trihydroxypregnane-3, 20-dione
Hydrocortisone
14α-Hydroxy-compound S
14α-Hydroxy-hydrocortisone
15α-Hydroxy-compound S
15β-Hydroxy-compound S
1-Dehydro-compound S
Δ4-Pregnene-17α, 20β, 21-triol-3-one
Androstene-dione
Testololactone
1-Dehydro-testolo-lactone
Compound S
Rhizopus nigricans
Curvularia lunata
Heliocostylum piriforme
Rhizopus nigricans
Rhizopus nigricans
Streptomyces fradiae
Cunninghamella blakesleeana
Helicostylum piriforme
Curvularia lunata
Hormodendrum viride
Spicaria sp.
C. simplex
D. lycopersici
Epicoccum oryzae
Aspergillus sp.
Penicillium sp.
Gliocladium sp.
Aspergillus sp.
Penicillium sp.
Cylindrocarpon radicicola

Fig. 4.5—Some microbiological transformations of Reichstein's compound S.

From Industrial Microbiology by S.C. Prescott & C.G. Dunn. Copyright 1959 by the McGraw-Hill Book Company, Inc. Used with permission of McGraw-Hill Book Company.

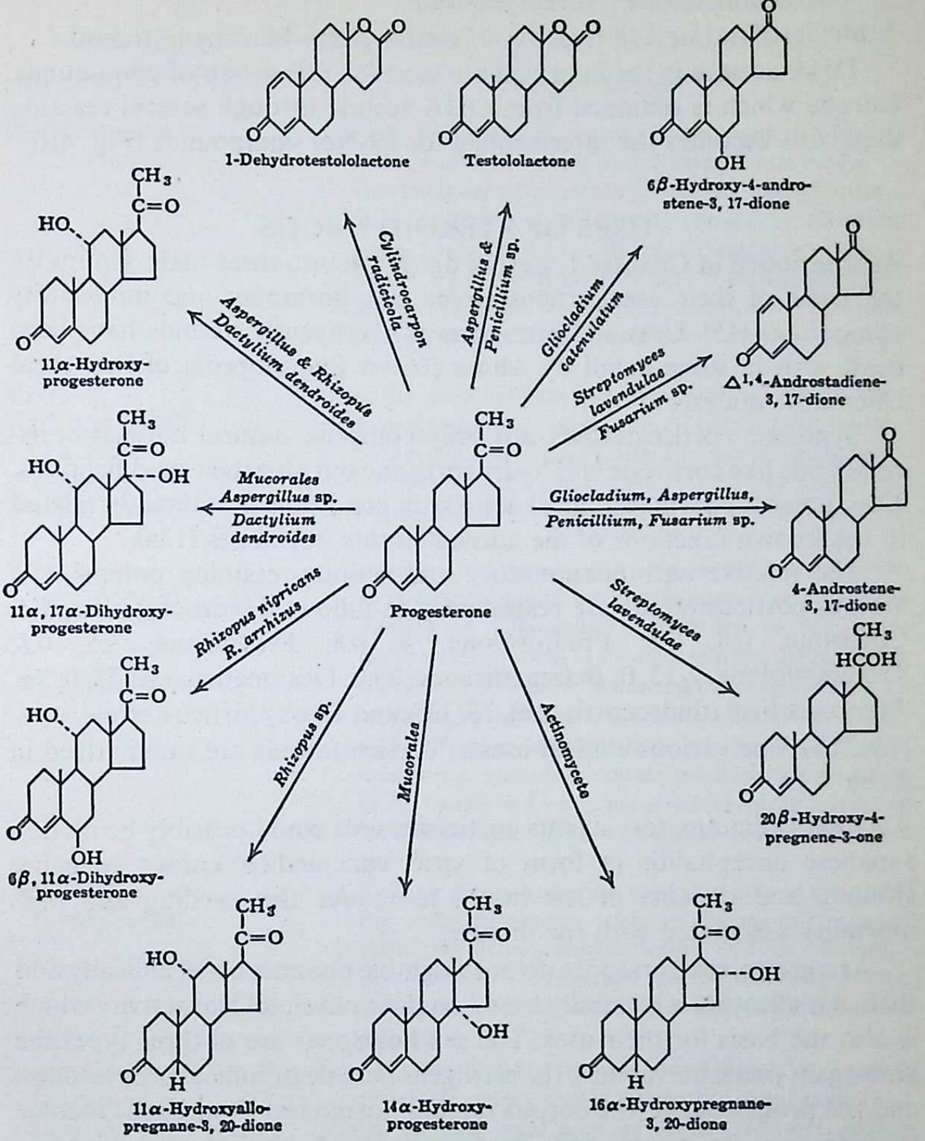

1-Dehydrotestololactone
Testololactone
6β-Hydroxy-4-androstene-3, 17-dione
11α-Hydroxyprogesterone
Cylindrocarpon radicicola
Aspergillus & Penicillium sp.
Gliocladium catenulatum
Aspergillus & Rhizopus
Dactylium dendroides
Streptomyces lavendulae
Fusarium sp.
Δ1,4-Androstadiene-3, 17-dione
11α, 17α-Dihydroxyprogesterone
Mucorales
Aspergillus sp.
Dactylium dendroides
Progesterone
Gliocladium, Aspergillus, Penicillium, Fusarium sp.
4-Androstene-3, 17-dione
Rhizopus nigricans
R. arrhizus
Streptomyces lavendulae
20β-Hydroxy-4-pregnene-3-one
6β, 11α-Dihydroxyprogesterone
Rhizopus sp.
Mucorales
Actinomyceta
11α-Hydroxyallopregnane-3, 20-dione
14α-Hydroxyprogesterone
16α-Hydroxypregnane-3, 20-dione

Fig. 4.6—Some microbiological transformations of progesterone.

From Industrial Microbiology by S.C. Prescott & C.G. Dunn. Copyright 1959 by the McGraw-Hill Book Company Inc. Used with permission of the copyright owner.

[Note—11β-Hydroxylation of compound S can be accomplished by *Cunnighamella blacksleena*, *Curvularia lunata*, *Streptomyces fradiae*, etc. to obtain hydrocortisone. *Corynebacterium simplex*, *Fusarium solanii*, etc. introduce Δ^1-double bond in hydrocortisone and cortisone (9, 12a).

Progesterone is converted to 11α-hydroxyprogesterone (yield 90% or more) by *Rhizopus nigricans* (12a).

Flavobacterium dehydrogenase does hydrolysis (of OAc) and oxidation (of 3β-OH group) with simultaneous rearrangement of double bond (Δ^5 to Δ^4)].

$\Delta^{5(10)}$-Norethisterone (Norethynodrel)
Ethinylestrenol or 17β-hydroxy-17α-ethinylestr-4-en (Lynestrenol).

DHA acetate is the intermediate used for this group of compounds. Estrone which is obtained from DHA acetate through several reaction steps also becomes the intermediate for 19-Nor compounds (Fig. 4.4).

USES OF STEROID DRUGS

As mentioned in Chapter 1, steroid drugs fall into three main groups on the basis of their use:Corticosteroids, sex hormones and antifertility compounds (15). Uses and properties of therapeutic steroids have been dealt with in some detail by Ahuja (16) in Encyclopedia of Industrial Chemical Analysis.

Synthetic corticosteroids not only comprise natural adrenal corticosteroids like cortisone and hydrocortisone but also their modifications. They possess pharmacological activity in conditions not directly related to the known functions of the adrenal cortex hormones (13a).

The relative anti-inflammatory and sodium-retaining potencies of various corticosteroids are respectively as follows: Hydrocortisone, 1,1; Cortisone, 0.8, 0.8; Prednisolone, 4, 0.8; Prednisone, 3.5, 0.8; Triamicinolone, 7-13, 0; Betamethasone, 25.0; Dexamethasone, 25, 0; 9α-Fluorocortisol (fludrocortisone), 15, 125; and Deoxycorticosterone, 0, — (13a, 16). The various clinical uses of corticosteroids are summarized in Table 4.1.

As anti-inflammatory agents corticosteroids could possibly be used in Japanese encephalitis (a form of virus encephalitis known to cause swelling and oedema of the brain) to reduce the swelling and high mortality associated with the disease.

As a group, sex hormones do not resemble one another chemically and their classification is generally based on their physiological activity which is also the basis for their uses. The sex hormones are of three types:the androgens (male hormones), the estrogens (female or follicular hormones) and the progestogens (the corpus luteum hormones). Androgens include testosterone, its esters and modifications. Anabolics are modified androgens with predominant growth-stimulant properties and with less of androgenic property (10). Estrogens include estrone, estradiol, and their esters and derivatives. Progestogens include progesterone, 17-α-hydroxyprogesterone derivatives and 19-Nor analogues. The uses of various sex hormones and their modifications are given in Table 4.2.

Antifertility compounds are mostly oral contraceptives in use today which are combination pills containing an estrogen (ethinyl estradiol and mestranol) and a progestogen (mostly 19-Nor compounds, and to a lesser extent derivatives of 17-hydroxyprogesterone).

(*Contd on page* 110)

TABLE 4.1—USES OF CORTICOSTEROIDS AND SPIRONOLACTONES

Corticoids	Actions & Uses[1,2,3,4]
Hydrocortisone (Cortisol) (a natural hormone; synthetic)	As anti-inflammatory agent; acetate salt in topical therapy of dermatoses; as succinate, intravenous or intramuscular administration in shock-like syndromes resulting from acute adrenal cortical insufficiency, acute hypersensitivity, status asthmaticus, disseminated lupus erythematosis and overwhelming infections; succinate and cypionate systemics; the latter (21-cyclopentane propionate ester) more pleasant in taste.
Cortisone (Compound E) (a natural hormone; synthetic)	Has the same actions and uses, but less potent than hydrocortisone; as acetate specifically used in combination with deoxycorticosterone acetate in adrenal cortical insufficiency; used also to suppress oversecretion of the adrenal cortex in bilateral hyperphasia.
Prednisolone (Modification)	A weaker mineralocorticoid than cortisol and cortisone; as butylacetate used for local injections or infiltration therapy of inflammatory disorders of the joints, tendons, and bursae; as 21-disodium phosphate also in the treatment of artheritedes and bursitides in the form of intrasynovial injection, and for inflammatory cysts and soft tissue inflammations in the form of local injection; prednisolone acetate, relatively non-irritating to the tissues and hence preferred for intramuscular or local injections, used where oral prednisolone not feasible.
Prednisone (Modification)	Actions similar to cortisone, used primarily in therapy of the collagen diseases such as rheumatoid arthritis, lupus erythematous,periarteritis nodosa, dermatomyositis and scleroderma and in the symptomatic management of allergic states especially bronchial asthma.
Betamethasone (Modification)	A glucocorticoid without mineralocortical action; extremely potent anti-inflammatory agent used.
Dexamethasone (Modification)	Same actions and uses as Betamethasone; sodium phosphate salt useful for intravenous administration being one of the most soluble corticoids.
Triamcinolone (Modification)	Nearly devoid of mineralocortical action; but the action may vary from person to person; used as anti-rheumatic agent.
Triamcinolone Acetonide (Modification)	Intended only for local application. Used in the treatment of inflammatory dermatoses, pruritis, arthritis, bursitis, tendonitis and synovitis, *not to be used* in infected areas of the skin without concomitant application of antibiotics.

(*Contd*)

Fludrocortisone (Modification)	As acetate has the same action and uses as hydrocortisone; as hemisuccinate (a water-soluble compound), used in opthalmology for treatment of allergic and inflammatory ocular and periocular disorders.
Deoxycorticosterone (natural & synthetic)	As acetate useful in treatment of Addison's disease.
Spironolactone[5] (modification)	Used to give a favourable diuretic action—release of sodium and water without loss of potassium in hyperproduction of aldosterone by the body. A member of series, aldactone is more potent and orally active compound causing sodium diuresis without potassium excretion.

[1] Travis & Sayers, *in* The Pharmacological Basis of Therapeutics, edited by Goodman & Gilman (The Macmillan Co., New York), 3rd ed., 1965, 1615-43.

[2] Ahuja *in* Encyclopaedia of Industrial Chemical Analysis, edited by Snell & Ettre (John Wiley & Sons, Inc., New York), Vol. 14, 1971, 191-99.

[3] Hardman, *Trop. Sci.*, 1969, **11**, 222-23.

[4] Butt, Hormone Chemistry (Ellis Horwood, Ltd., Chichester), 2nd (revised) ed., Vol. 2, 1977, 174-80.

[5] Kirk-Othmer Encyclopaedia of Science & Technology (Interscience Publishers, a division of John Wiley & Sons, Inc., New York), 2nd ed., Vol. 18, 1969, 869-70.

TABLE 4.2—USES OF SEX HORMONES AND THEIR MODIFICATIONS[1,2,3,4]

Sex Hormones	Actions & Uses
Androgens	
Testosterone & Esters (natural hormone; synthetic)	Employed as substitutional therapy in male deficiencies or in men or youth with impaired or destroyed testicular function; also employed to facilitate development of adult masculine characteristics in delayed adolescent process; useful in therapy of patients with hypopituitarism or with Addison's disease; in the treatment of postpartum breast engorgement and for suppression of lactation; in the palliative treatment of breast cancer in women who are premenopausal or early post menopausal; in uterine disorders and nostalgia. Esters are much less polar and when injected in oil, are absorbed more slowly. Propionate is much more active than free testosterone; longer acid esters are longer acting.
17α-Methyltestosterene	Actions and uses same as testosterone, but orally effective.
Methandienone (modification)	An androgenic steroid with relatively strong anabolic and weak androgenic activity; used mainly to promote nitrogen anabolism and weight gain in cachexia and debilating diseases and after serious infections, burns, trauma, or surgery.
19-Nortestosterone (modification)	Phenylpropionate of 19-nortestosterone called nandrolone phenpropionate used as an anabolic.

(*Contd*)

Estrogens	
Estrone (natural hormone; synthetic)	Used for stimulation of development of uterus or of secondary characteristics in women with delayed or inadequate development, and in treatment of dysmenorrhea and other gynecological disorders; as substitution therapy when menopausal symptoms occur after cessation of ovarian function following castration by X-rays or radium therapy; also used as an intermediate for 19-norcompounds.
Estradiol & Esters (natural hormone; synthetic)	Replacement therapy in estrogen deficiency; also employed in prostatic carcinoma and inoperable mammary carcinoma especially in postmenopause; in the treatment of gynecological disorders. The esters more superior and more commonly used to supplement parenteral therapy since estradiol itself is rapidly destroyed.
Ethinyl estradiol (modification)	Actions and uses as those of the other estrogens; ethimyl radical delays the decomposition of the estradiol molecule that occurs during absorption by oral route; one of the most potent estrogens known; used in oral contraceptives; also used in skin cream and lotions for the improvement in skin tone.
Mestranol (modification)	It is a methyl ether of ethinyl estradiol; readily absorbed by the oral route; inhibits the release of pituitary gonadotropins and as such useful for suppressing ovulation in the treatment of endometriosis; used in oral contraceptives.
Pregnenolone[5] (non-hormonal natural compound; synthetic)	Estrogen-like effects on the skin without any systemic effects; as acetate, used in cosmetic cream to counteract wrinkles by hydration and plumping of the skin; used more as an intermediate than as finished product.
Progestogens	
Progesterone (natural hormone; synthetic)	Employed in various gynecological disorders, habitual abortion, menopausal syndrome, infertility; also employed in prostatic carcinoma, acne vulgaris.
17α-Caproxyloxyprogesterone (a derivative)	Highly active subcutaneously; used as long acting progestational agent since slowly transported from the site of injection; virtually inactive by mouth.
17α-Acetoxypyrogesterone (a derivative)	Highly active progestational agent by both the oral and subcutaneous routes; employed to a small extent in oral contraceptives, an intermediate for powerful analogues like 6α-methyl and 6-chloro substituted compounds given below.
Medroxyprogesterone & Chlormadinone acetates (derivatives)	Analogues of 17α-acetoxy progestestrone, they are 6α-methyl and 6-chloro substituted compounds; extremely active progestational agents; chlormadinone acetate possesses an oral progestational activity about 500 times greater than 17α-acetoxyprogesterone and approximately 35 times greater than 19-norethisterone.
Ethisterone (modification)	The same uses as progesterone. *(Contd)*

Norethisterone (modification)	A progestational agent with weak estrogenic and androgenic actions; used in oral contraceptives; weak androgenic properties may cause deepening of the voice, hirsuitism and acne, and may cause masculinization of fetus.
Norethynodrel (modification)	Isomeric with norethisterone; shift of the double bound from 4(5) position to 5(10) position abolishes androgenic properties; employed for progestational therapy or for oral contraception along with an estrogen to prevent withdrawal bleeding and to enhance the anovulatory effect.
Ethinyl estrenol (Lynestrenol)	A powerful progestational agent free of endrogenic and anabolic effects; used in oral contraceptives and gynecological disorders.

[1] Astwood, *in* The Pharmacological Basis of Therapeutics, edited by Goodman & Gilman (The Macmillan Co., New York), 3rd ed., 1965, 1540-65, 1572.

[2] Ahuja, *in* Encyclopaedia of Industrial Chemical Analysis, edited by Snell & Ettre (John Wiley & Sons, Inc., New York), Vol. 14, 1971, 199-205.

[3] Zderic, *in* Comprehensive Biochemistry, edited by Florkin & Stotz (Elsevier Publishing Company, New York), Vol. 10, 1963, 181, 188-91.

[4] Butt, Hormone Chemistry (Ellis Horwood, Ltd., Chichester), 2nd (revised) ed., Vol. 2, 1977, 118-46; 60-80; 81-117.

[5] Applezweig, Steroid Drugs (McGraw-Hill Book Co., Inc., New York), 1962, 277.

Some of the wellknown contraceptive pills have the following combinations with milligram dosages in parentheses: Norethynodrel - Mestranol (10.0 - 0.15, 5.0 - 0.075, 2.5 - 0.1); Norethindrone - Mestranol (2.0 - 0.1); Norethindrone acetate - Ethinyl estradiol (2.5 - 0.05); Medroxyprogesterone acetate - Ethinyl estradiol (10.0 - 0.05, 5.0 - 0.05, 2.0 - 0.02); 6-Dehydromedroxyprogesterone - Ethinyl estradiol (4.0 - 0.05); Ethinyl estrenol - Mestranol (5.0 - 0.15); and *dl*-Norgestrel - Ethinyl estradiol (0.3 - 0.03). *dl*-Norgestrel is a totally synthesized progestogen (3 ab).

REFERENCES

1. Applezweig, N. Steroid Drugs (McGraw-Hill Book Co., Inc., New York), 1962, 53-83.
2. Fieser, L.F. & Fieser, M. Steroids (Reinhold Publishing Corporation, New York), 1959, 549-50, 694; Topics in Organic Chemistry, 1963, 256-57.

3a. Hamied, Y.K. Progress and prospects of the steroid industry in India (Lecture delivered at the Indian Institute of Science, Bangalore on March 8, 1976); 3b. Information from Dr. Y.K. Hamied, Managing Director, CIPLA, Bombay.

4. Chakravarti, R.N., Chakravarti, D. & Mitra, M.N. Isolation and reactions of steroid sapogenins. *J. Indian Chem. Soc.*, 1961, **38**, 635-41.
5. Dauben, W.G. & Fonken, J.G. Isomerization of isospirostans to furostenols with pyridine hydrochloride as the catalyst. *J. Am. Chem. Soc.*, 1954, **76**, 4618-19.

6. Cameron, A.F.B., Evans, R.M., Hawlet, J.C., Hunt, J.S., Jones, P.G. & Long, A.G. Synthesis of cortisone. XII. Improvements in the conversion of sapogenins into pregnan-20-one. *J. Chem. Soc.*, 1955, 2807-16.

7. Kunjithapadam, S. Utilization of various raw materials for the production of steroids, *in* Cultivation & Utilization of Medicinal and Aromatic Plants, edited by Atal, C.K. & Kapur, B.M. (Regional Research Laboratory, Jammu-Tawi), 1977, 9-20.

8. Kent, J.A., Beesch, S.G. & Tanner (Jr), F.W. Industrial fermentation processes, *in* Riegel's Industrial Chemistry, edited by Kent, J.A. (Reinhold Publishing Corporation, New York), 1962, 216-17.

9. Prescott, S.C. & Dunn, C.G. Industrial Microbiology (McGraw-Hill Book Co., Inc., New York), 3rd ed., 1959, 723-61.

10. Hardman, R. Pharmaceutical products from plant steroids. *Trop. Sci.*, 1969, **11,** 196-228.

11. Butt, W.R. Hormone Chemistry, Vol. 2—Steroids, Thyroid Hormones, Biogenic Amines and Prostagladins (Ellis Horwood Ltd., Chichester), 2nd (Revised) ed., 1977, 147-90.

12a Ringold, H.J. & Bowers, A. Adrenal hormones, *in* Comprehensive Biochemistry, Vol. 10—Sterols, Bile Acids and Steroids, edited by Florkin, M. & Stotz, E.H. (Elsevier Publishing Co., New York), 1963, 81-165; 12b. Zderic, J.A. Progestational hormones, *in* Comprehensive Biochemistry, Vol. 10, 166-96.

13a. Travis, R.H. & Sayers, G. Adrenocorticotropic hormone; adrenocortical steroids and their synthetic analogs, *in* The Pharmacological Basis of Therapeutics, edited by Goodman, L.S. & Gilman, A. (The Macmillan Co., New York), 3rd ed., 1965, 1608-48; 13b. Astwood, E.B. Androgens, *in* Pharmacological Basis of Therapeutics, 1572-73.

14. Kirk-Othmer Encyclopedia of Chemical Technology. (John Wiley & Sons, Inc., New York), 2nd ed., Vol. 18, 1969, 845-46, 849.

15. International Trade Centre UNCTAD/GATT Markets for Selected Medicinal Plants and their Derivatives. Geneva, 1974. Chapter IV—Dioscorea and Diosgenin, 69-71.

16. Ahuja, S. Hormones: Steroids, *in* Encyclopedia of Industrial Chemical Analysis, edited by Snell, F.D. & Ettre, L.S. (John Wiley & Sons, Inc., New York), Vol. 14, 1971, 191-205.

CHAPTER 5

STEROID DRUG PRECURSORS OTHER THAN DIOSGENIN

Diosgenin has been the steroid drug precursor of choice the world over till recently. The situation started changing in the seventies when rising prices and uncertainty of the availability of *Dioscorea* tubers caused shortage of steroid drugs based on diosgenin. The demand for these drugs, however, kept on growing. These factors turned the attention of steroid drug producers to other precursors and a search was on for new and cheaper sources. The development of an alternate route of microbiological fermentation which was more efficient than conversions by chemical means gave further impetus to the use of base materials other than diosgenin. As a consequence, precursors like sterols and hecogenin, particularly the former, have come into prominence and taken the place of diosgenin in many of the uses. The use of diosgenin is now restricted to manufacture of corticosteroids and here too stigmasterol, solasodine and hecogenin are making inroads. However, in India, the steroid drug industry is still based almost entirely on diosgenin.

The steroid drug precursors other than diosgenin fall into the following two categories: vegetable sources like solasodine, hecogenin, stigmasterol, sitosterols and ergosterol; and animal sources such as cholesterol, and deoxycholic and cholic acids (the bile acids). Systematic chemical names of these precursors along with their properties are given in Table 5.1.

Non-steroidal precursor like squalene, which can be converted to cholesterol or to AD and ADD by fermentation, is not dealt with here.

SOLASODINE

Of the other steroid precursors, solasodine, N-analogue of diosgenin (Fig. 1.1), is currently the most promising in India. Solasodine is present in the form of its glycosides in several genera belonging to the *Solanaceae* and *Liliaceae* families. The genus *Solanum* forms the most important source (1, 2). Several glycosides of solasodine combined with different sugar moities are known (3).

Solasodine or for that matter *Solanum* group of alkaloids as a whole have been subject of extensive investigation both for their chemistry as well as for their distribution in the plant kingdom in order to discover new and more convenient sources of steroid precursors. These investigations have been reviewed by Schreiber (3).

Solasodine is obtained from the leaves of *Solanum aviculare* Forst. f., and from the ripe fruits of *S. khasianum* Clarke and its variety *chatterjeeanum* Sen Gupta (= *S. viarum* Dunal)* (1). In *S. aviculare*, it is present as solasonine (1 rhamnose, 1 galactose and 1 glucose) and solamargine (2 rhamnose and 1 glucose); in *S. khasianum* and its variety, it is present as solasonine (2). Commercial processing of the leaves of cultivated *S. aviculare* is reported in Hungary and Russia; according to Applezweig (4) production has been stopped. Several other countries are examining the prospects of obtaining solanum alkaloids by developing suitable *Solanum* species (4, 5).

In India, Glaxo Laboratories (India) Ltd. have started production of solasodine (2.5 tonnes per annum) since August 1976 from *Solanum khasianum* var. *chatterjeeanum* (6, 7a). Of the several solasodine-containing *Solanum* species (Table 5.2), *S. khasianum* var. *chatterjeeanum* is preferred in India, since it is widely distributed and its fruits are reported to be a richer source of the alkaloid than the leaves of *S. aviculare* (2). *S. khasianum* is equally valued; it may be mentioned here that some workers do not distinguish between *S. khasianum* and its var. *chatterjeeanum*.

Solanum khasianum Clarke

It is a native of India and is found in the Khasi and Jaintia Hills of Meghalaya, Naga Hills of Nagaland, Manipur, and Arunachal Pradesh up to an altitude of 1,850 m (2).

Var. *chatterjeeanum* grows wild in the Khasi and Jaintia hills of Meghalaya, Arunachal Pradesh, Sikkim, West Bengal, Orissa, Gangetic plains and in the Nilgiris, ascending to an altitude of 1,600 m (2).

Wild *S. khasianum* is a stout, much branched perennial undershrub up to 1.5 m tall, with almost straight prickles. Leaves are ovate, lobed, lobes lanceolate or triangular, hirsute and prickly on both surfaces; flowers white, in lateral 1-4 flowered racemes; berries yellowish or greenish, globose, 2.5 cm diam.; seeds smooth brown compressed (2).

---

*According to Babu, C.R. (*J. Bombay Nat. Hist. Soc.*, 1970, **67**, 609-11) variety *chatterjeeanum* Sen Gupta is in fact homologous to the south Brazilian plant, *Solanum viarum* Dunal, in all important characteristics and as such the latter has a priority over it in nomenclature. In his opinion, this plant is a chance introduction in India and has got widely naturalized in several states. The name *S. viarum* is gaining wide circulation. However, Deb, D.S. (*Solanaceae Newsl.*, No. 2, 1975, 33-34) claimed to have traced valid nomenclature to *S. myriacanthum* Dunal. He has suggested that the name takes precedence over others viz., *S. reflexum* Schrank, *S. platanifolium* Hook., *S. viarum* Dunal, *S. khasianum* and its variety *chatterjeeanum*, which may be treated as its synonyms.

TABLE 5.1—SYSTEMATIC CHEMICAL NAMES AND PROPERTIES OF STEROID PRECURSORS OTHER THAN DIOSGENIN

Precursor	Systematic chemical name[1]	Formula[2]	Mol. wt[2]	M.p.°C[2,3]		*$[\alpha]^{\circ}_{D}$[2,3]	
				Precursor	Acetate	Precursor	Acetate
Solasodine	25 *R*, 22α-Spirosol-5-en-3β-ol	$C_{27}H_{43}O_2N$	413	202	195 141-42 (picrate)	−92.4 (benzene)	—
Hecogenin	3β-Hydroxy-25 *R*, 5α-spirostan-12-one	$C_{27}H_{42}O_4$	430	268**	245**	+ 10	− 1
Stigmasterol	Stigmast-5, 22 *E*-diene-3β-ol	$C_{29}H_{48}O$	412	170	144	− 51	− 56
β-Sitosterol	Stigmast-5-en-3β-ol	$C_{29}H_{50}O$	414	140	134 (MeOH)	− 37	− 38.5
Cholesterol	Cholest-5-en-3β-ol	$C_{27}H_{46}O$	386	149 (EtOH)	114-15	− 39	− 47.4
Deoxycholic acid	3α, 12α-Dihydroxy-5β-cholan-24-oic acid	$C_{24}H_{40}O_4$	392	176-77 (EtOH)	105-06 (Me ester)	+ 53 (EtOH)	+ 48 (MeEt ketone)
Cholic acid	3α, 7α, 12α-Trihydroxy-5β-cholan-24-oic acid	$C_{24}H_{40}O_5$	408	196-98	155 (162) (Me ester: 2 forms)	+ 37 (EtOH)	+ 30.1 (+ 25) (Me ester: 2 forms)
Ergosterol	Ergosta-5,7,22 *E*-triene-3β-ol	$C_{28}H_{44}O$	396	163 (Et_2O-EtOH)	175-76 (Et_2O-EtOH)	− 133	—

[1] Cumulative Chem. Abstr. Index Buide, 1972-76, **76-85**. [2] Dictionary of Organic Compounds, edited by Heilbron and others, 4th ed., 1965, Vol. 5,2804-05; 2918; 2902; 3137; Vol. 3, 1809; 1350-51, Vol. 2, 702; 1063. [3] Rodd's Chemistry of Carbon Compounds, edited by Coffey, S., 1971, 2nd ed., Vol. II, Part E 1971, 26; Vol. II, Part D, 1970, 164-66; 172-73.

*Unless otherwise stated the solvent is chloroform.

**According to Dictionary of Organic Compounds, hecogenin occurs in 3 polymorphs with m.ps 268°, 245° and 253°.

TABLE 5.2—SOLASODINE CONTENT OF DRIED FRUITS OF SOME INDIAN SOLANUM SPECIES

Species	Solasodine content, %
S. aviculare Linn. (leaves)[1,*]	1.80-3.42
S. elaeagnifolium Cav.[2]	1.50-2.07
S. incanum Linn.[2]	1.80-2.00
S. indicum Linn.[2]	1.20-1.80
S. khasianum Clarke[2]	1.00-1.75
var. *chatterjeeanum*[3]	0.50-5.40
S. laciniatum Ait.[4,*]	2.12-3.06
S. mammosum Linn.[2,5,*]	0.80-1.25
S. nigrum Linn.[1,2]	0.00-1.05
S. platinifolium Sims.[6]	1.93
S. robustum E. Wendl.[1,*]	1.02
S. surattense Burm. f.[1,2]	1.10-2.08
S. trilobatum Linn.[2]	3.50†
S. verbascifolium auct. non Linn.[2]	0.01-3.82

[1] Gadwal, Paper presented at NBG Symposium, Lucknow, March, 1977.

[2] Kaul & Zutshi, *in* Cultivation & Utilization of Medicinal and Aromatic Plants, 1977, 26.

[3] Puri & Bhatnagar, *Pharmacos*, 1974, **19**, 7-24.

[4] Bordoloi *et al.*, *Indian Drugs*, 1976, **14**(1), 5-11.

[5] Upadhyay *et al.*, Indian J. Pharm., 1976, **38**, 52-53.

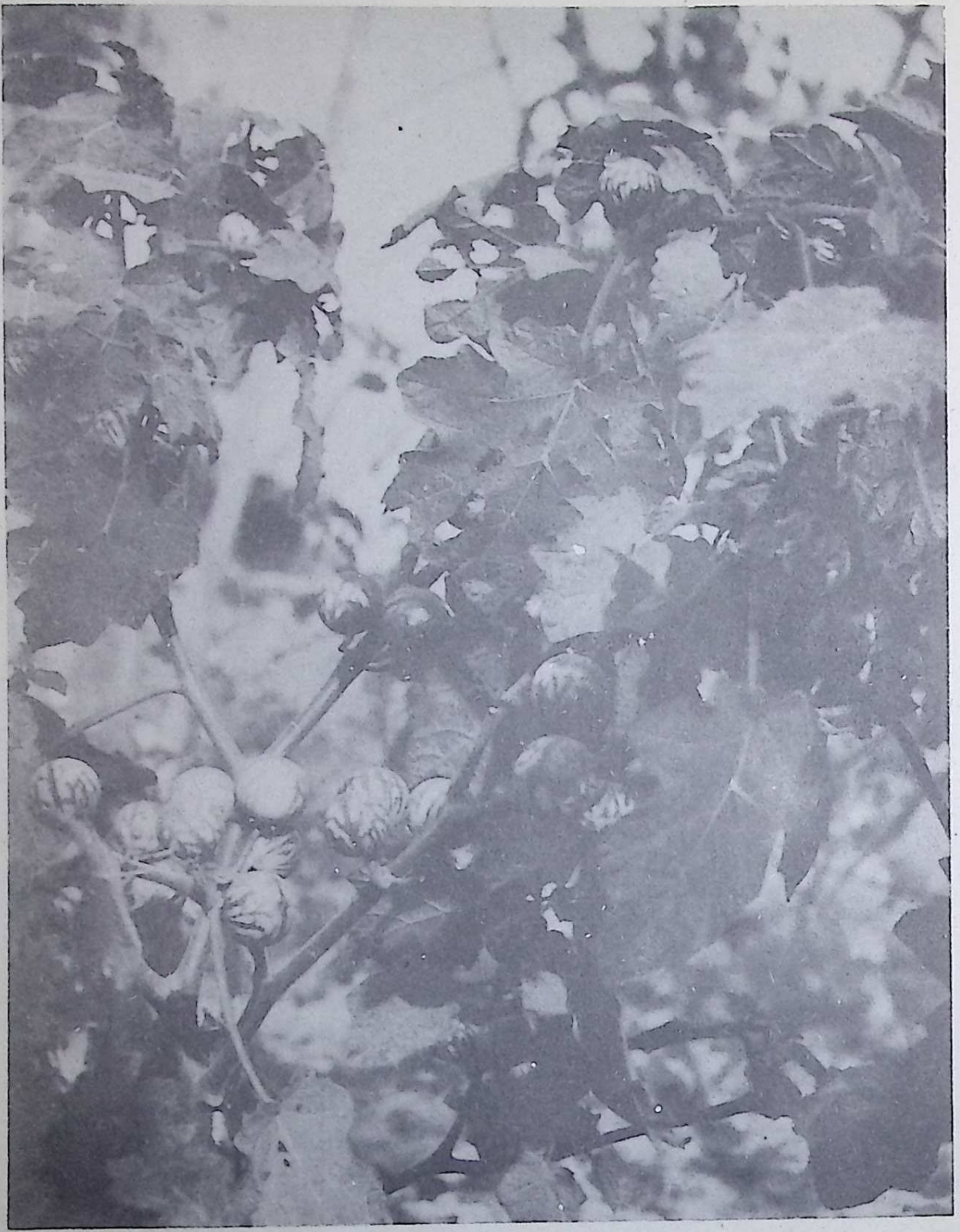
[6] Puri & Bhatnagar, *Lloydia*, 1974, **37**, 318-19.

*Introduced; †Total glycoalkaloids.

Var. *chatterjeeanum* is an erect, much branched undershrub or shrub, with prickles of two types on the stem; straight, and distinctly recurved. Leaves are large, with deltoid or ovate lobes, prickly on both surfaces; and flowers white or pale yellow (2), see Figs. 5.1 and 5.2.

The wild berries show wide variation in solasodine content, ranging from less than 0.5 per cent to 5.4 per cent on dry weight basis (8, 9, 10). The alkaloid content is maximum when the colour of the berries changes from green to yellow (2). It varies from region to region and with the age of the plant; also, more the number of seeds in the fruit, higher the alkaloid content (11). Solasodine is concentrated in the mucilage surrounding the seeds (2), but for commercial production, dried whole fruits are preferred.

S. khasianum and its variety *chatterjeeanum* are distributed almost all over India, but the wild plants could not be depended upon for isolation of solasodine because of the wide variations in the yields of berries and of the alkaloid. Concerted research efforts have therefore been directed towards development of cultivated species showing improvements over the wild in the following respects:



Dr. M.C. Tyagi, Genetics, IARI, New Delhi

Fig. 5.1—*Solanum khasianum* var. *chatterjeeanum*.

1. Minimum of spines or prickles to make hand-picking of fruits easy,
2. Increase in number of berries per plant,
3. Higher solasodine content, preferably above 3 per cent, and
4. Synchronous maturity of fruits for maximum yield of solasodine and therefore, synchronous flowering and fruiting.

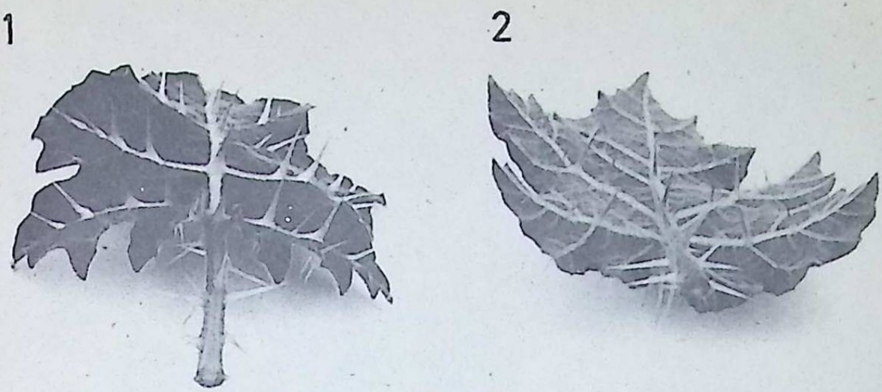
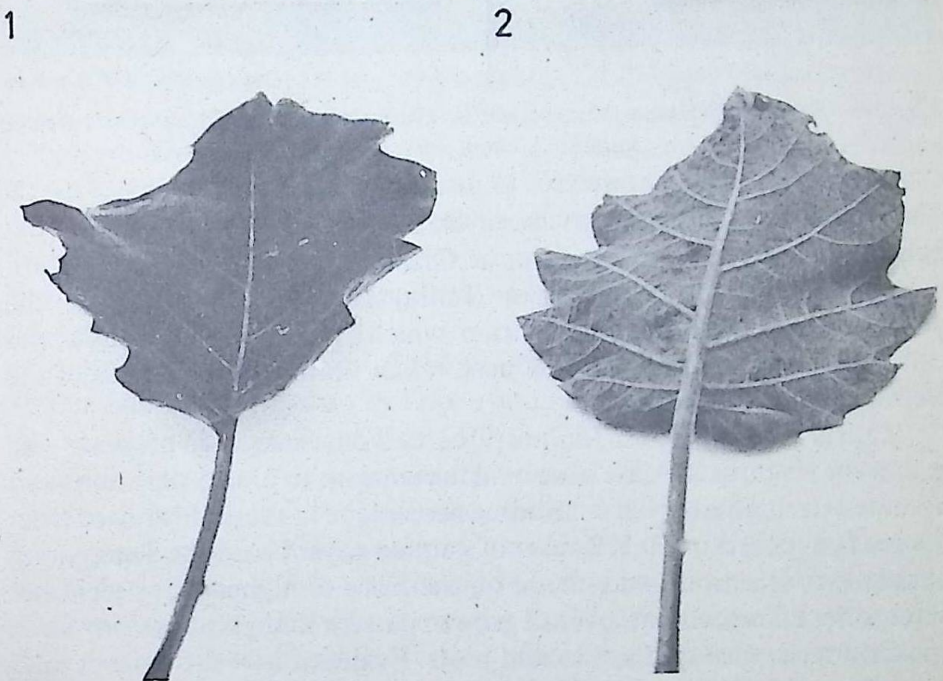


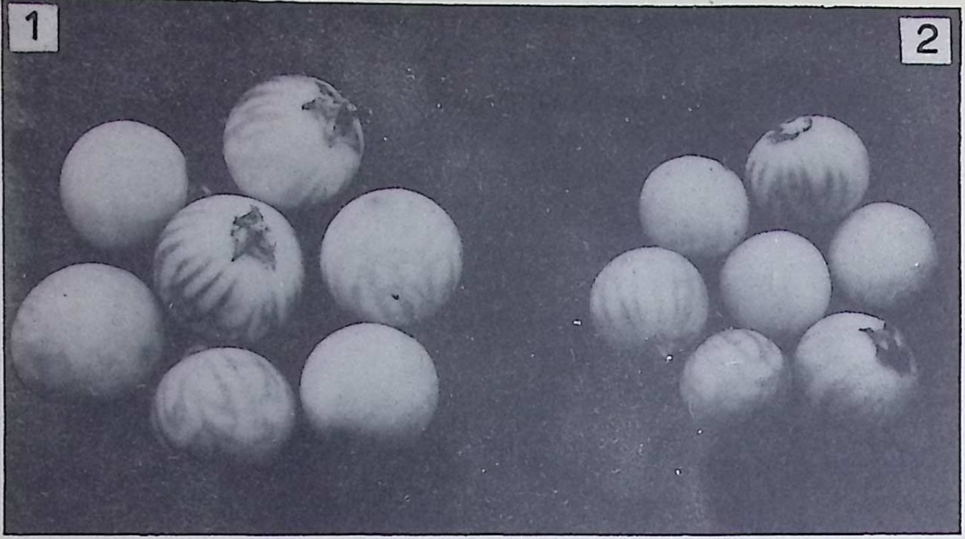
Fig. 5.2—Leaf of *Solanum khasianum* var. *chatterjeeanum:* 1, Ventral view; 2, Dorsal view.



Dr. M.C. Tyagi, Genetics, IARI, New Delhi

Fig. 5.3—Leaf of reduced-spine mutant of *Solanum khasianum* var. *chatterjeeanum*: 1, Ventral view; 2, Dorsal view.

Genetic Improvement—A large collection of germplasm was made and usual breeding methods like hybridization and polyploidy having failed, breeding through induced mutation was resorted to by several investigators (9, 10, 12a, 13). Use of gamma rays as well as chemical mutagens such as ethylmethane sulphonate (EMS), ethylenimine (EI), nitrosoguanidine (NG) and N-nitro-N-methylurea (NMU) was made to evolve mutants with reduced spine (Fig. 5.3) and yielding higher number of berries of bigger size and higher solasodine content (Fig. 5.4).



Dr. M.C. Tyagi, Genetics, IARI, New Delhi

Fig. 5.4—Fruits of ***Solanum khasianum*** var. *chatterjeeanum:* 1, From NMU-treated mutant; 2, From normal plant.

Glaxo has been interested in breeding a spineless plant type *S. khasianum* var. *chatterjeeanum* since 1965. Extensive breeding and agronomic researches carried out at Glaxo Research Ltd., Sefton Park (U.K.) and Glaxo Laboratories (India) Ltd. have resulted in the development of the improved strain which is now under commercial cultivation. Extension work has been taken up in different parts of the country (7a).

Kaul & Zutshi of RRL, Jammu (9) carried out estimation of solasodine and berry yields in the case of several mutants up to four generations and obtained the highest mean solasodine percentage in the plants raised from the seeds exposed to 10 KR dose of gamma rays. From the segregating population, selections were made on the basis of higher berry yield per plant, solasodine content, overall growth pattern and plant performance, and field resistance to diseases and pests. Evaluation of the mutant lines on the basis of yield potential in well-laid statistical designs led to a strain named RRL-20-2, which showed promise as high berry and high solasodine (2.5-3.0%) yielding plant (9).

An interselection of cross JRL-13 × JRL-26 developed by RRL, Jorhat is reported to yield more than 1 kg of berries per plant with solasodine content up to 2.77 per cent (14a).

Prominent among the improved strains that have been evolved are: (i) Glaxo strain, (ii) RRL-20-2 of RRL, Jammu, and (iii) JRL strain of RRL, Jorhat, and (iv) Reduced spine mutant of IARI (see Fig. 5.3) (6, 7a, 9, 10, 12a, 14a). All these strains can be grown as annuals. Improved strains have also been developed by RRL, Bhubaneswar.

Seed Supply—Glaxo Laboratories supply seeds after testing for their germination percentage to interested parties each season. Age of the seed is an important factor to reckon with because viability is lost in about 2 months (7a). A limited amount of seeds of RRL-20-2 strain are available at a nominal price for commercial cultivation (9). Seeds of JRL strain of RRL, Jorhat are sold to interested parties (14a). The seeds of IARI reduced spine mutant are also under consideration for commercial release (12b). However, Glaxo strain is reported to surpass the other three strains in yield potential in terms of berries as well as solasodine content though not in overall field resistance to diseases and pests. A number of drug firms as well as growers of medicinal plants are now showing interest in commercial cultivation of this crop. The price offered by Glaxo is Rs 2 per kilogram of dry berries containing 1 per cent solasodine (9).

Packages of practice for commercial cultivation of Glaxo strain and RRL-20-2 strain of *S. khasianum* or its variety are summarized in Table 5.3. Although two crops a year can be taken, Dr. Gadwal (7c) recommends a good rotation with a leguminous crop.

Area under Cultivation and Yields—Area under cultivation of Glaxo strain has increased from 62 hectares in 1971 to over 600 hectares in 1977 (7c). Its cultivation is concentrated in the following districts: Mahbubnagar in Andhra Pradesh; Baroda and Bulsar in Gujarat State; Raichur, Hassan and Bangalore in Karnataka; and Jalgaon in Maharashtra.

Large scale cultivation of the improved strain developed by RRL, Jorhat has been taken up in Diphu in collaboration with Assam Hills Development Corporation (14a).

Cultivation of *S. khasianum* in tea gardens of North Bengal is underway from the seeds distributed by the West Bengal Pharmaceutical & Phytochemical Development Corporation. The Corporation sells seeds at Rs 400 a kg., but seeds could be had at half the price by giving an undertaking to sell the berries to the said Corporation (15). The area under cultivation is likely to increase considerably due to the popularity this easily-grown crop enjoys. According to Applezweig (5), the cultivation of solanum plants is certain to grow in countries of cheap labour, wishing to have indigenous source for steroid drugs.

Under average care, yield of 10,000 kg of fresh berries (moisture content, 82%) and under better conditions 15,000 kg per hectare per season can be obtained from Glaxo strain; solasodine content varies from 2 to 4 per cent.

The berries are dried soon after harvesting to avoid fungal attack and metabolic degradation (9).

Solasodine Extraction—Of the several methods available for isolation of solasodine, Bakshi & Hamied's method (16) is the most suitable. The

TABLE 5.3—CULTIVATION PRACTICES FOR GLAXO STRAIN AND RRL-20-2 STRAIN OF S. KHASIANUM

	Glaxo strain[1]	RRL-20-2 Strain[2]
Crop	From selected seeds	From selected seeds
Planting	Seedling transplanting preferred	Seedling transplanting give better results
Seedling time	1½ to 2 months for seeds to germinate in nursery ry	—
Duration of crop	6 Months in the field	6 Months in the field
Climate & soil	The monsoon of north India and areas where rainfall is not very heavy as in Thana (*c.*250cm) are suitable. Very severe winter not conductive	A vigorous and hardy plant, can grow on any soil under various agro-climatic conditions but generally prefers moderate climate for full growth
Spacing	90 cm × 150 cm (90 cm within the rows and 150 cm between the	50 cm × 50 cm
Irrigation	Assured water supply in all the seasons for irrigation	Immediate irrigation after transplantation followed by once a week for one month, thereafter once a fortnight
Weeding	—	First weeding after 3-4 weeks Second weeding after 2-3 months
Fertilizers	A fertilizer rate of 36 kg N; 36 kg P_2O_5 and 18 kg K_2O in two doses	50 kg/ha each of super phosphate, potash and urea dose when the plants have taken stand and repeat dose at the time of flowering
Diseases and pests	Subject to a number of diseases and pests. Wilting caused by *Fusarium oxysporum*, a major disease	No major disease or pests. Aphid attack at seedling stage, which can be overcome by malathion sprays

[1] Gadwal, *in* Cultivation & Utilization of Medicinal & Aromatic Plants, 1977, 31-33.
[2] Kaul & Zutshi, ibid., 23-31.

method involves ethanolic extraction of glyco-alkaloids from dry powdered berries, followed by treatment with hydrochloric acid, precipitating solasodine-hydrochloride which on basification with ammonia solution releases solasodine. The alkaloid is purified by crystallization from acetone. Pure solasodine has m.p. 202° (Table 5.1).

The fatty oil in the berries may cause interference in isolation procedure. Kaul & Zutshi (9) have modified the above method by first defatting the powdered berries with petroleum ether and isolating the alkaloid more or less by Bakshi & Hamied's method. Petroleum ether extract of the berries of RRL-20-2 yields a greenish yellow fatty oil (8-10%) which can be used as a by-product.

It is reported that unlike the berries of RRL-20-2 strain, those of Glaxo strain contain only traces of fatty oil and hence there is no interference in extraction of the alkaloid (9, 17). It is not known though, how Glaxo's procedure avoids the formation of 3:5 diene. The machinery used for solasodine extraction differs from that used for diosgenin extraction.

Problems—As a newly established crop, there are a number of problems encountered in its cultivation which are summarized below (7a, 9):

1. Under optimum conditions of temperature and moisture, the germination of the seeds start after 8-10 days and continue till 30-45 days. The problem is to ensure almost all the seedlings at one time, especially where a farmer has small acreage.

2. Newer pests and diseases, hitherto unrecorded on this crop, have been reported with the establishment of this species as a crop and its large scale cultivation; *Pests*—Fruit borer, root eating grubs, leaf eating caterpillars and leaf hoppers; *Diseases*—leaf spot disease in Karnataka, virus symptom (not on big scale), wilting caused by *Fusarium oxysporum* (7a). Glaxo Laboratories are facing difficulties in controlling the soil borne disease of wilt caused by *F. oxysporum* and have not been able to isolate any donor for resistance. This is the most important areas where the plant breeders and plant pathologists in the country can fruitfully work in collaboration to find a solution to this problem (7a). The fungicides, bavistin, agallol, cerasan and aureofungin have been found to control the wilt disease effectively (14a). RRL-20-2 strain is disease-free comparatively (9). In this connection mention may be made that intra-specific hybridization between stock of various mutants with desirable economic characters may prove useful (12b).

3. Drying of the fresh berries during the monsoon season poses a big problem. Drier with hot air is being considered for quick drying.

4. There is lack of information on several aspects of agronomy like optimum plant population, levels of nutrients, and effect of fertilizer on solasodine content, etc.

5. Synchronous maturity of fruits for maximum solasodine production is still far from reality.

Germplasm collection—According to Gadwal (7b), a plant exploration trip was undertaken in the north eastern parts of the country covering regions around Darjeeling (West Bengal), Khasi hills (Meghalaya), and Jenabil in Mayurbhunj forests (Orissa) with an objective of developing a less spiny, high yielding plant type of *S. khasianum* var. *chatterjeeanum.* Considerable variation in plant heights, plant types, size of the leaves and intensity of spininess (2 mm to 20 mm) as also in the average weight of a single berry (1.20 to 11.0 g) and in solasodine content (0.75 to 3.96%, dry wt basis) was observed. Glaxo Laboratories are maintaining the germplasm and have used them in breeding for resistance to the wilt, but without any success so far. Collections from Jorhat (Assam) and Nilgiris (Tamil Nadu) are also being screened.

Economics of S. khasianum Cultivation

According to Dr. Gadwal (7c), to get an average yield of fresh berries (moisture content, 82%) of say about 10 tonnes per hectare per crop from Glaxo strain with 2.5% of solasodine, total expenses come to about Rs 3,750/- and profits per hectare per crop would be approximately Rs 5,000/-. In actual practice, there is considerable variation in the management of individual farms and that is reflected in the cost of cultivation. Kaul & Zutshi of RRL, Jammu have worked out the economics of cultivation of the strain, RRL-20-2, envisaging a profit of about Rs 5,560 per hectare.

Comparison with Dioscorea floribunda—Production statistics of *solanum* berries and solasodine from Glaxo strain and of the tubers and diosgenin from *D. floribunda* FB(C-1) strain, grown around Bangalore, are compared in Table 5.4.

In the first year, solasodine yield per hectare can be equal to that of diosgenin with the assumption that 2 harvests a year are available;

TABLE 5.4—COMPARATIVE YIELDS OF SOLASODINE AND DIOSGENIN

	S. khasianum var. *Chatterjeeanum* (Glaxo strain)[1] Berries	*D. floribunda* [FB(C-1) Strain][2] Tubers
Fresh wt of crop kg/ha/year	10,000-15,000/season 20,000-30,000/year (82% moisture)	Min. 25,000 kg (1-year crop, 70% moisture)
Dry wt of harvest kg/ha/year	3,600-5,400	7,500
End-product content, %, dry wt basis	2-4 Solasodine	2.5-3.0 Diosgenin
End-product yield kg/ha/year	72-216 Solasodine	187.5-225 Diosgenin
End-product yield kg/ha/2 years	144-432 (4 crops in 2 years)	495-540 kg (2-year crop, 3% diosgenin)

[1] Gadwal, *in* Cultivation & Utilization of Medicinal & Aromatic Plants, 1977, 31-33.

[2] Bammi & Randhawa, Dioscorea Status Report, 1975, 51-52. See also Table 3.1.

however when *D. floribunda* is grown as 2-year of 3-year old crop (as is the practice of one drug company), tuber yield multiplies from 2 to 5 times and diosgenin content increases considerably, thereby widening the gap between solasodine production and diosgenin production during the same period. Furthermore, the yield of diosgenin is generally more than is assumed here for calculation purposes, and also in reality only a single crop of solanum per year may be harvested.

Profits—Assuming that two crops of *S. khasianum* are harvested per year the profit expected is round Rs 10,000 to 11,000 per hectare which is comparable to the profit of Rs 10,000 to 12,500 from 1-year old crop of *D. floribunda* (18). But if 2-year old crop of *D. floribunda* is harvested then the profit is in the range of Rs 25,000 to 30,000 whereas profit in 2 years for *Solanum* berries will be Rs 20,000 to 22,000. If the 3-year old crop of *D. floribunda* is harvested, then expenses being nominal on cultivation in the third year and tuber weight increasing enormously, the profit will be much more as compared to *Solanum* berries during the same period (6 crops). Nevertheless, initial expenses on cultivation of *Solanum* species are nominal as compared to *Dioscorea* cultivation, and that makes the cultivation of this plant popular amongst the farmers. Furthermore, *Solanum* is a short duration crop which can flourish on a land where fastidious *Dioscorea* cannot be grown (12a).

Cost of Solasodine—Although Glaxo have started production of solasodine, they are still in the process of optimizing the extraction conditions (17). It is, therefore, not possible to compare the cost of extraction of 1 kg of solasodine with that of 1 kg of diosgenin.

Other Solanum Species

A number of indigenous *Solanum* spp. contain 1 per cent or more of solasodine (Table 5.2). Of these, *S. elaeagnifolium* Cav. which is spineless has received some attention as a source for the collection of fruits and extraction of solasodine. It requires 100-120 days to give a crop as against 1·80 days for *S. khasianum.*

S. aviculare and *S. laciniatum* Ait. (earlier treated synonymously with *S. aviculare*) (14bcde) which are non-spiny plants where fruits as well as leaves yield solasodine, have been introduced into India. There are some reports of successful cultivation of these species by RRL, Jammu (2, 19) and RRL, Jorhat (14c): of these *S. laciniatum* introduced from New Zealand is being multiplied. The problem according to Gadwal (7b) appears to be that of limited adaptability as these have to be tried in temperate conditions. However, a single plant of *S. aviculare* grown through stem cuttings at Thana (Maharashtra State) by Glaxo showed a range of 1.8 to 3.4 per cent of solasodine in leaves in four months of duration; seeds posed a germination problem.

All the three species are spineless type giving 3 to 4 crops a year involving no harvesting hazards; they merit further attention as alternative crops for production of solasodine (20).

Prospects for Solasodine

At present solasodine appears to be a stop-gap precursor for Indian steroid industry, but with further improvement in existing strains of *S. khasianum* var. *chatterjeeanum* as also development of other *Solanum* species with 3 to 4 crops a year, solasodine may hold its own against diosgenin. Cultivation of improved strains can give boost to rural economy. *Solanum* spp. have the advantage over dioscoreas in that they can be grown easily and rapidly in areas of cheap labour. However, according to diosgenin manufacturers in India, solasodine will remain a supplementary source of steroid drugs.

In the world market, prices of both diosgenin and solasodine are falling down and both are losing ground. The fact that both diosgenin and solasodine are the precursors obtained from plants which are specially grown for these without any by-products, makes it difficult for them to compete with other precursors which are all by-products of some industry or the other and hence are becoming important. With accent on recycling of wastes all over the world, prospects for solasodine are rather bleak.

REFERENCES TO SOLASODINE

1. Blunden, *et al.* *Trop. Sci.*, 1975, **17**, 139-54.
2. The Wealth of India—Raw Materials, Vol. IX, 1972, 379-429.
3. Schreiber, K. Steroid alkaloids: the *Solanum* group, *in* The Alkaloids, edited by Manske, R.H.F. (Academic Press, Inc., New York), X, 1968, 1-192.
4. Applezweig. Dioscorea — the pill crop, 1976.
5. UNCTAD/GATT Markets, 1974, 79-80.
6. Information from Dr W.G.E. Underwood, Technical Director, Glaxo Laboratories (India) Ltd, Bombay.

7a. Gadwal, V.R. Commercial cultivation of *Solanum* crop (Glaxo Strain), *in* Cultivation & Utilization of Medicinal and Aromatic Plants, 1977, 31-33; 7b. Cultivation of *Solanum* species for the production of solasodine — problems and prospects. (Paper presented at NBG, Lucknow Symposium, held on March 26-28, 1977); 7c. Information from Dr V.R. Gadwal, Head, Botany R&D, Glaxo Laboratories (India) Ltd., Thana, Maharashtra.

8. Puri, R.K. & Bhatnagar, J.K. Studies on the Genus *Solanum* — a review. *Pharmacos*, 1974, **19**, 7-24.
9. Kaul, B.L. & Zutshi, U. Cultivation of *Solanum khasianum* Clarke for Steroids: problems and promises, *in* (see reference 7a). 23-31.
10. Kaul, B.L. The place of *Solanum khasianum* among steroid-bearing plants. Proc. Second Workshop of AICIP on Medicinal and Aromatic Plants held at Anand, on November 1-4, 1976, 192-94.
11. Saini, A.D. & Safri, B. Studies with *Solanum* species as a source of solasodine. *Indian J. Genet*, 1974, **34A**, 1210-12.

12a. Tyagi, M.C., Sharma, B. & Jain, H.K. Introduction of economically valuable mutations in *Solanum viarum*.Proc. Second Workshop of AICIP on Medicinal and Aromatic Plants, held at Anand, on November 1-4, 1976, 219-25; 12b. Information from Dr M.C. Tyagi, Scientific Officer, IARI, New Delhi.

13. Chauhan, Y.S., Singh, K.K. & Ganguli, D. Gamma-ray-induced variations in some quantitative characters in *Solanum khasianum* Clarke. *Indian Drugs*, 1975, **13**(3), 17-19.

14a. *Annu.-Rep. RRL.Jorhat*, 1975 & 1976, 24, 58; 14b. 1969-70, 23; 14c. 1967-68, 42; 14d. 1970-71, 41; 14e. 1973, 33.

15. *Data India*, May 23-29, 1977, No.21, 238.

16. Bakshi, V.M. & Hamied, Y.K. Isolation of solasodine from *Solanum khasianum* grown in Bombay. *Indian J. Pharm.*, 1971, **33**, 54-55.

17. Information from Shri M.K. Pradhan, Manager, R&D, Glaxo Laboratories (India) Ltd., Worli, Bombay.

18. Bammi, R.K., Rao, G. & Randhawa, G.S. Commercial cultivation of *Dioscorea floribunda*. Extension Ser. No.9 (Indian Institute of Horticultural Research ICAR, Bangalore), 1975.

19. Chandra, V. Production of *Solanum* species for steroidal hormones. *Indian Drugs Annu.*, 1968, 57-67.

20. Gupta, R. Crop improvement in *Solanum viarum* Dunal with particular reference to proposals for improvements (Paper presented at NBG, Lucknow Symposium, held on March 26-28, 1977).
(see also Reference page 145).

HECOGENIN

Hecogenin is used for the manufacture of corticosteroids, and accounts for about 6 per cent of the steroid precursors presently being used by the world pharmaceutical industry. It is a steroidal sapogenin (Fig. 1.3), present in the plants in the form of its glycoside, heconin (1, 2). Hecogenin occurs in the sap of several *Agave* spp., which are also grown for their fibres commonly known as sisal fibre. Besides *Agave*, hecogenin is also present in a few *Yucca* species (3, 4) and *Hechtia texensis* S. Wats. (5).

Hecogenin is commercially obtained from sisal juice, a by-product of sisal fibre (1). It is mainly produced in Tanzania, Kenya, and Israel from *Agave sisalana* Perr. (sisal), and in Mexico from *A. rigida* Mill syn. *A. fourcroydes* Lem. (henequen) (2, 6, 7). Hybrids with high hecogenin content relatively free from tigogenin (with which it is generally associated) have been developed elsewhere (8). Haiti is reported to be the most promising new area for the production of hecogenin (9).

Sisal wastes are of two types: (i) the expressed juice obtained by dry decortication of fresh leaves without any chemical treatment, and (ii) the residue along with washings, left after the removal of the fibre from the leaves (10, 11). Of these wastes, the former makes a more attractive source material for the extraction of hecogenin (11). The solid residue yields a hard lustrous wax with high melting point, which is a good substitute for carnauba wax (12), an imported item in India (10).

Attempts are being made in India to exploit sisal waste material for the economic recovery of hecogenin (11). The patented technical knowhow for converting this genin or its intermediate into corticosteroids, is available with multinational companies like Glaxo and Merck, Sharpe & Dohme operating in India.

The National Committee on Science and Technology has recommended the constitution of a Working Group to prepare a status report on sisal waste (13) on the following points: Availability of sisal waste regionwise and the possibility of its centralised collection; status of technology and the future research needs; import-export position of wax and hecogenin.

Four *Agave* species. are gorwn in India for sisal fibre; these are *A. sisalana* commonly known as sisal, *A. cantala* Roxb. known as Cantala or Bombay Aloe, *A. vera-cruz* Mill and *A. angustifolia* Maw. syn. *A. wightii* Drummond & Prain (14). According to estimates made by Indian Institute of Foreign Trade, the total area at present under sisal in the country is 2,500 hectares (13). *Agave* spp. are mainly grown in Andhra Pradesh, Assam, Bihar, Karnataka, Maharashtra, Orissa, Tamil Nadu, and West Bengal (10, 15). Available data on area under cultivation of *Agave* spp. and production of juice in some states are summarized in Table 5.5; figures for individual species are not available. Plantations are managed both by public and private sectors.

Yield—Amount of juice varies from 15 to 25 per cent of the green weight of the leaves. Average weight of a sisal leaf is about 900 g, and 1,000 leaves yield 27 to 36 kg of dry fibre and 135 to 225 kg of juice (16).

Yields of hecogenin varying from 0 to more than 1 per cent have been reported elsewhere (4). A group of workers (7) from National Botanic Gardens, Lucknow have investigated hecogenin contents of leaves of

TABLE 5.5—AREA UNDER SISAL CULTIVATION AND ESTIMATED PRODUCTION OF JUICE IN SOME STATES

State	Area in hectares	Juice in tonnes
Andhra Pradesh[1]	1011*	—
Bihar[2]	73	9.1
Maharashtra[1]	209*	—
Orissa[2]	1200	150.0
West Bengal[2]	430	50.0

[1] Saraf, *et al.*, *Ind. Bull., Maharashtra*, 1966, 7(2), 1.

[2] Information obtained from Sisal Research Station, Bamra, through Dr. S.N. Mahapatra, RRL, Bhubaneswar.

*These figures are based on 1966 report.

Note : Data from Assam, Karnataka and Tamil Nadu are not available.

Agave species grown by Sisal Research Station, Bamra (Orissa). The results of their findings are summarised in Table 5.6. Hecogenin content of leaves shows seasonal variations (3, 11) and increases with the age of the plant (4, 12).

Investigations carried out at the Indian Institute of Science, Bangalore show that sisal waste from *A. vera-cruz* grown in South India is a good

TABLE 5.6—HECOGENIN CONTENTS OF THE LEAVES OF SOME AGAVE SPECIES[1]

Agave spp.	Hecogenin content % dry wt basis
A. ameniensis Trelease	0.05
A. americana Linn.	0.04 (0.065)†
A. angustifolia Haw.	0.02
A. cantala Roxb.	0.06 (0.15)‡
A. chieslereghlu	0.10
A. decipiens Baker	0.16
A. elongata Jacobi	0.12
A. hybrid	0.11
A. mirasdorensis Jacobi	0.03
A. nirvana	0.10
A. sisalana Perr.	0.03 (0.10)‡
A. vera-cruz Mill	0.03
A. versehaftelu	0.03

[1] Dixit *et al.*, *Indian J. Pharm.*, 1974, **36**, 119-20; figures are for species grown at Sisal Research Station, Bamra.

†Singh & Periera, *Indian J. Chem.*, 1964, **2**, 297; sample from Saugar, Madhya Pradesh.

‡Chakravarti *et al.*, *Bull. Calcutta Sch. Trop. Med.*, 1958, **6**, 60; samples from West Bengal.

TABLE 5.7—HECOGENIN CONTENT OF SISAL WASTES FROM AGAVE SPECIES

Agave spp.	Place	Hecogenin content of wastes %
A. cantala	Calcutta[1]	0.5 (sludge)
A. sisalana	Calcutta[1]	0.25 (sludge)
	Calcutta[2]	1.1-1.3 (sludge)
	Calcutta[2]	1.5-2.0 (pulp)
	Nildumri[3]	0.1 (solid wastes)
	Nildumri[4]	0.1 (juice)
A. vera-cruz	Bangalore[5]	0.1 (juice)

[1] Chakravarti *et al.*, *Bull. Calcutta Sch. Trop. Med.*, 1962, **10**, 3-4.

[2] *Annu. Rep. Jute Technol. Res. Lab.*, 1971, 11.

[3] Srinivasulu & Mahapatra, *Res. & Ind.*, 1971, **16**, 183-4.

[4] Information from Dr S.N. Mahapatra, RRL, Bhubaneswar.

[5] Subba Rao & Shyama Sunder, *Indian J. Chem.*, 1974, **12**, 429.

source of hecogenin, absolutely free from tigogenin; press-juice obtained from the leaves during various seasons do not seem to contain even traces of tigogenin (17, 18).

Sisal wastes available from commercially grown *Agave* species in India is estimated at 10,000 tonnes/annum. About 1,500 kg of hecogenin can be recovered from these wastes after drying. Glaxo Laboratories are producing 120 kg of corticosteroids such as cortisone, prednisone and betamethasone; at this production level, they require more than 2,500 kg of hecogenin as the sole starting material (10). Hecogenin content of sisal wastes (juice/sludge) obtained from various places in India is given in Table 5.7.

Extraction of Hecogenin

The wastes obtained from sisal industry can yield hecogenin by the same conventional or standard methods as are employed for the extraction of diosgenin. However, commercial exploitation of sisal waste is beset with certain difficulties, namely:

1. Hecogenin is associated with tigogenin in *Agava* species and the content of hecogenin and the ratio of the two genins vary widely with the season of the year and the age of the plant.
2. The juice tends to form slimes difficult to filter even after acid treatment, making mechanical operations very difficult.
3. In the normal decortication process large amount of water is added, resulting in enormous volume of liquid effluents which have to be concentrated before extraction. Concentration of the liquid effluents requires large amount of fuel.

In view of the above, it is clear that for extraction of hecogenin dry decortication method should preferably be used for the expression of sisal leaf juice. The fleshy waste material or the leaf pulp remaining after decortication of sisal is expressed by passing through heavy serrated rollers. The juice thus obtained is allowed to ferment for more than 24 hours, whereby a partial enzymatic hydrolysis of heconin takes place resulting in precipitation of the hemisaponin (still retaining part of the sugar moiety) in the form of a fine sludge which slowly settles down to the bottom of the tank. The clear supernatant liquid is discarded, and the sludge treated with mineral acid (HCl or H_2SO_4) for hydrolysing the hemisaponin to the genin. The mixture is filtered and washed with water and the resulting brown cake is treated with methanolic sodium hydroxide to remove residual acid as well as other impurities. A light-brownish granular solid, obtained on filtration and washing, is crystallized from toluene, ethyl acetate or other suitable solvent to give substantially pure hecogenin (4).

Several modifications to this process have been suggested. One modification covered by British patent (19) employs 2 per cent sulphuric acid instead of enzymatic hydrolysis in the above technique for the precipitation of hemisaponin as a fine sludge (about 0.5% of the original volume) which is refluxed with 10 per cent acid to hydrolyse the hemisaponin completely. Acid from the second treatment is recycled for the preliminary partial hydrolysis of next batch of juice. The double acid treatment reduces the total amount of acid required at the plantation without decreasing the ease of handling and filtration at various stages.

Another modification covered by U.S. Patent (20), which aims at elimination of complicated apparatus and the necessity of using chemicals at the raw material site; appears to have an edge over others. Partially hydrolysed heconin (and other saponins) can be advantageously separated by air floatation method; fermented sisal juice (48 ml) separates into two phases—the liquid and the cloudy sediment phase. The former is drawn off and discarded, while the latter (13 ml) is pumped into a vertical tank. Air is blown for 2 hours through a jet pipe at the bottom of the tank and the solution allowed to stand. Clear liquid, 4 ml, is drained off from the bottom. This process is repeated and 2 ml more of clear liquid are removed. The resulting thick slurry is then taken out and dried by hot air. The dried material can be hydrolysed by usual method at any convenient location. The yield by this method is claimed to be twice the amount of sapogenin obtained by the conventional method. Floatation and wetting agents can be added to help the separation at air floatation level.

A method developed by Regional Research Laboratory, Bhubaneswar (15) more or less employs the conventional technique whereby hecogenin is obtained from sisal juice by concentration, hydrolysis with acid, followed by solvent (hydrocarbon solvent) extraction, in an yield of about 0.1 per cent. The problem of slime or resin formation is claimed to have been overcome in this method and purification made simpler. This process is ready for commercial exploitation. It can process 0.5 tonne of juice per day to get 0.5 kg of hecogenin. According to Glaxo, a capacity of 10 kg/day of hecogenin acetate would be required for an economically viable unit. For this purpose 10 tonnes of sisal juice in dry condition would be required per day.

Jute Technological Research Laboratories, Calcutta have reported hecogenin yield as high as 1.1 to 1.3 per cent in pure form from the sludge obtained from the fermented leaf juice of *A. sisalana* (21ab). Hecogenin was obtained by hydrolysing the sisal sludge (solid content 17%) with 3N sulphuric acid on a boiling water bath, filtering, washing, drying and then extracting with petroleum ethers. Crude genin obtained by concentration of extract was crystallized from benzene, after decolourizing with crude

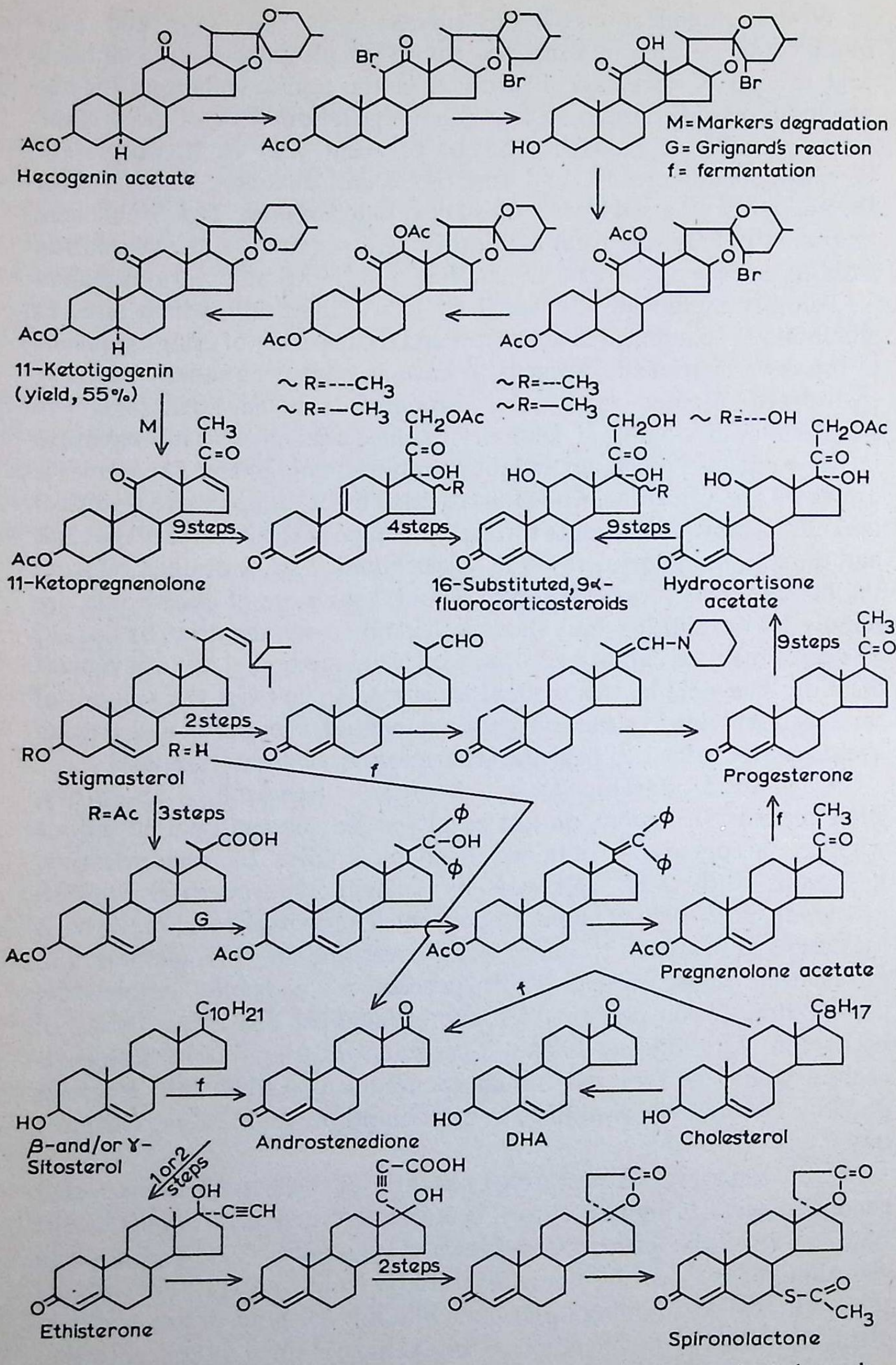

Hecogenin acetate
M=Markers degradation
G= Grignard's reaction
f= fermentation
11-Ketotigogenin
(yield, 55%)
M
~R=---CH3
~R=—CH3
~R=---CH3
~R=—CH3
~R=---OH
9steps
4steps
9steps
11-Ketopregnenolone
16-Substituted, 9α-fluorocorticosteroids
Hydrocortisone acetate
9steps
Stigmasterol
2steps
R=H
f
Progesterone
R=Ac
3steps
G
f
Pregnenolone acetate
f
f
β-and/or γ-Sitosterol
Androstenedione
DHA
Cholesterol
1or2 steps
2steps
Ethisterone
Spironolactone

Fig. 5.5—Molecular flow-sheet of some steroid processes using hecogenin and sterols.

charcoal, to give hecogenin m.p. 247°. The crude material may also be acetylated to give hecogenin acetate, m.p. 244° (see also Table 5.1).

Prospects for Hecogenin

Hecogenin, because of its additional C-12 oxygen function (Fig. 1.1), is not as versatile as diosgenin, and can be used only in one group of steroids, namely corticosteroids. In India, there seems to be little likelihood that manufacturers other than those having patents or technical knowhow will switch over from using diosgenin to hecogenin (22). There is a possibility that one of the multinational companies operating in India which has the know-how may start using hecogenin as the starting material (13, 10).

Conversion to various corticoids from hecogenin has been dealt with in several works in detail (4, 2). See Figs. 5.5 and 5.7.

REFERENCES TO HECOGENIN

1. Blunden, *et al.* *Trop. Sci.*, 1975, **17**, 139-54.
2. Fieser & Fieser. Steroids, 1959, 667-671; Topics in Organic Chemistry, 1963, 259-60.
3. Callow, R.K., Cornforth, J.W. & Spensley, P.C. A source of hecogenin. *Chem. & Ind.*, 1951, 699-700.
4. Applezweig. Steroid Drugs, 1962, 47-49, 66-70, 83.
5. Dictionary of Organic Compounds (Eyre & Spottiswoods Publishers Ltd., London), 4th ed., 1965, Vol.3, 1809.
6. Guillebaud, C.W. An Economic Survey of the Sisal Industry of Tanganyika (James Nisbet & Co. Ltd., Welwyn, England), 3rd ed., 1966, 1, 44.
7. Dixist, B.S., Khanna, R.K., Srivastava, S.N. & Kapoor, L.D. Isolation of hecogenin from some *Agave* species. *Indian J. Pharm.*, 1974, **36**, 119-20.
8. UNCTAD/GATT Markets, 1974, 79.
9. Applezweig. Dioscorea—the pill crop, 1976.
10. *CSIR News*, 1971, **21**, 15.
11. Information through personal communication or discussion.
12. Lock, G.W. Sisal—Twenty-five Years' Sisal Research (Longmans, Green and Co. Ltd., London), 1962, 313.
13. Draft Status Report on Utilization & Recycling of Wastes (Research, Development and Extension Requirements) (National Committee on Science and Technology, Government of India, New Delhi), 1975, 124.
14. The Wealth of India — Raw Materials, Vol. I, 1948, 38-40.
15. Mhaskar, V.V., Hinge, V.K. & Shah, S.M. Wax from sisal waste. *Res.& Ind.*, 1959, 4, 219-20.
16. Information from Dr. S.N. Mahapatra, Scientist, RRL, Bhubaneswar.
17. Export Potential of Selected Medicinal Plants and their Derivatives (Basic Chemicals, Pharmaceutical & Cosmetics Export Promotion Council, Bombay), 1976, 50.
18. Subba Rao, G.S.R. & Shyam Sunder, N. Steroidal sapogenins from *Agave vera-cruz* Mill. *Indian J. Chem.*, 1974, **12**, 429.
19. *Chem. Abstr.*, 1956, **50**, 2689; 1955, **49**, 15184.
20. *Chem. Abstr.*, 1965, **62**, 6348.

21a. *Annu. Rep. Jute Technol. Res. Lab., Calcutta*, 1971; 21b. Information from Shri A.C. Chakravarty, Director, Jute Technological Research Laboratory, Calcutta.
22. Manning. *Trop. Prod. Inst. Rep.*, No.G 41, 1969, 9.
(see also Reference page 145).

STIGMASTEROL

Stigmasterol (Fig. 1.1) is used by only one company, Upjohn in the USA, as a starting material for conversion into progesterone, and is estimated to represent about 15 per cent of the total steroid precursors used (1).

Stigmasterol is widely distributed in plants but its principal sources are the calabar or ordeal bean (*Physostigma venenosum* Salf.) from which it derives its name, and soybean oil (*Glycine max* Merril syn. *G. soja* Sieb. & Zucc.) (2, 3ab). The main supplier of calabar bean is Sierra Leone. The bean is used in medicine for its alkaloid, physostigmine, which is official in I.P. and B.P. *P. venenosum* is reported to be introduced into India (3a).

Soybean is an important crop in the Far East, the U.S.A. and many other countries. Stigmasterol is recovered in the U.S.A. as a by-product of soap stock or foot from alkali refining of soybean oil. Sterol mixtures obtained from the unsaponifiable matter (foot) contain 25 per cent of stigmasterol; the remainder consists of β- and γ-sitosterols the latter predominating in the sterol mixture (4).

Extraction—Sterol mixtures are recovered from the foot by treating it with calcium oxide and extracting the resulting porous granular mass continuously with acetone which leaves behind objectionable gummy and waxlike materials. The crude mixture obtained on evaporation of the solvent can be purified by a low-temperature crystallization from alcohol.

For separation of stigmasterol from the closely related sistosterols, the sterol mixture is first acetylated by conventional method and then brominated in acetic acid till all the double bonds are saturated. The stigmasterol acetate tetrabromide is separated by crystallization from alcohol or other suitable solvent. Pure stigmasterol can be obtained from the tetrabromide by treatment with zinc dust, saponification, and crystallization from acetone. Sterols or their lower fatty acid ester can be separated by crystallization using a hydrocarbon or chlorinated hydrocarbon. Widely different solubilities of α-naphthylcarbonates of stigmasterol and sitosterols can also be taken advantage for their separation (5).

Soybean has been introduced into India and its cultivation is reported in a number of states (3b). Large-scale recovery of stigmasterol from soybean oil was recommended at the Symposium on 'Production and Utilization of Vegetable Raw Materials for Steroid Hormones and Oral Contraceptives' held in March 1977, at NBG, Lucknow. Soybean oil industry is still in its infancy in India and it remains to be seen if some steroid drug manufacturers take up the isolation of stigmasterol (6a).

Among the other possible sources of stigmasterol in India, mention may be made of the stems and leaves of *Laptadenia reticulata* Wight & Arn., (Hindi, *Dori*), a shrub of common occurrence in India, yield 0.04 per cent of stigmasterol in free form. A simplified procedure for the isolation of stigmasterol has been described; vitamin E (tocopherols) can be obtained as a by-product in a yield of 0.2 per cent (7, 3c). Lablab bean (*Dolichos lablab* Linn.) and Cowpea (*Vigna unguiculata* Walp) contain 0.027 and 0.025 per cent of stigmasterol respectively, but unlike soybean, the oil content of both the pulses is very small (8).

Conversion of stigmasterol—It is relatively easy to convert stigmasterol into steroid hormones; because of the double bond in the side chain, it is amenable to degradation. However, it is not easy to obtain the pure crystalline stigmasterol needed for the purpose (9).

Stigmasterol is converted to pregnenolone acetate which is subjected to a fermentation process that does the hydrolysis, oxidation and rearrangement to produce progesterone in excellent yields. Upjohn (U.S.A.) uses the fermentation process and obtains progesterone from 500 kg batches of pregnenolone acetate (6a). See Fig. 5.5 and Fig. 5.6.

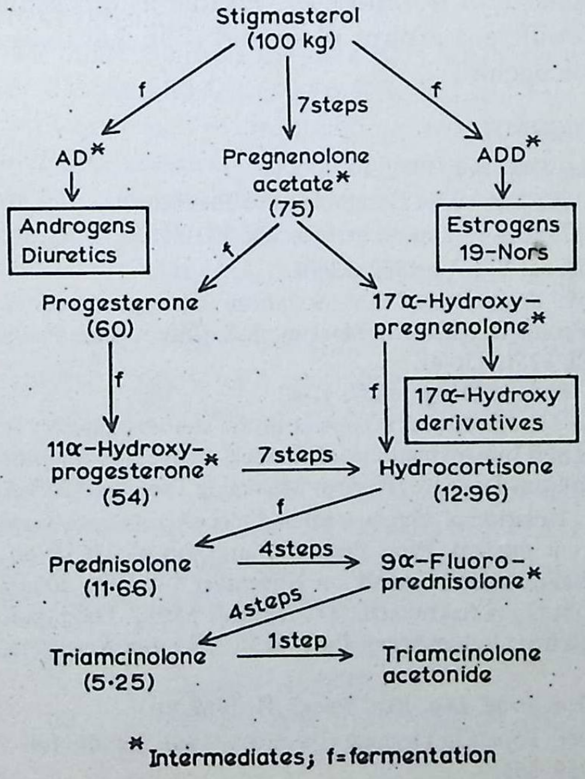

Stigmasterol
(100 kg)
f
7steps
f
AD*
Pregnenolone acetate* (75)
ADD*
Androgens
Diuretics
Estrogens
19-Nors
f
Progesterone (60)
17α-Hydroxy-pregnenolone*
f
17α-Hydroxy derivatives
f
11α-Hydroxy-progesterone* (54)
7steps
Hydrocortisone (12·96)
f
Prednisolone (11·66)
4steps
9α-Fluoro-prednisolone*
4steps
Triamcinolone (5·25)
1step
Triamcinolone acetonide
*Intermediates; f=fermentation

Fig. 5.6—Product pattern with intermediates and yields from stigmasterol.

Another efficient route to progesterone, developed by the same company (Fig. 5.5), does not involve pregnenolone as an intermediate. Stigmasterol is first converted to stigmastadienone by Oppenauer oxidation, the dienone in methylene chloride containing 1% by volume of pyridine is then subjected to ozonization, and the resulting aldehyde is treated with piperidine in the presence of a catalytic amount of *p*-toluenesulfonic acid to give enamine, which is oxidised with sodium dichromate in anhydrous acetic acid-benzene to yield progesterone (10a). The overall yield of progesterone by this route is 61 per cent from stigmasterol (10b). As mentioned in Chapter 1, this process forced the prices of Syntex progesterone down from $ 480/kg to $ 190/kg. However, with the development of fermentation process, pregnenolone route has gained an upper hand.

Stigmasterol can be convered to androstenedione (AD) and androstadienedione (ADD) through microbiological fermentation (Table 5.8). Sterol mixture from soybean (containing stigmasterol and sitosterols) could possibly be directly fermented to AD and ADD with a common micro-organism, eliminating altogether the separation step for the production of these intermediates. Being a by-product precursor, the stigmasterol-based steroid drugs are cheaper than those based on diosgenin. Stigmasterol is almost as versatile as diosgenin and can be converted into different groups of steroid (Fig. 5.6) unlike cholesterol, sitosterol, or hecogenin.

REFERENCES TO STIGMASTEROL

1. Blunden, *et al. Trop. Sci.*, 1975, **17**, 139-54.
2. Kritchevsky, D. Sterols, *in* Comprehensive Biochemistry, Vol. 10, 1963, 13.

3a. The Wealth of India — Raw Materials, Vol. VIII, 1969, 41-42; 3b. Vol. IV, 1956, 142-50; 3c. Vol. VI, 1962, 73-74.

4. Lehman, R.W. & Embree, N.D. Soyabean oil byproducts, *in* Soyabean and Soyabean Products, edited by Markley, K.S. (Interscience Publishers, Inc., New York), Vol.2, 1951, 836-41.
5. Applezweig. Steroid Drugs, 1962, 41-42.

6a. Hamied, Y.K. Progress and prospects of the steroids industry in India (Lecture delivered at the Indian Institute of Science, Bangalore, on March 8, 1976); 6b. Information from Dr Y.K. Hamied, Managing Director, CIPLA, Bombay.

7. Anjaria, J.V. Isolation of stigmasterol and tocopherols from *Leptadenia reticulata* by a short cut method. Proc. Second Workshop of AICIP on Medicinal and Aromatic Plants, held at Anand, on November 1-4, 1976, 206-07.
8. Chakravarti, R.N., Chakravarti, D., Mitra, M.N., Dasgupta, B. & Maiti, P.C. Sterols from Indian beans. Part I & II. *J. Sci. Ind. Res.*, 1956, **15C**, 86-88; 89-90.
9. Manning. *Trop. Prod. Inst. Rep.* No. G 41, 1969, 10.

10a. Fieser & Fieser. Topics in Organic Chemistry, 1963, 260-61; 10b. Fieser & Fieser. Steroids, 1959, 556.

(see also Reference page 145).

SITOSTEROLS

Sitosterols have come into prominence because of their easy microbiological conversion to AD and ADD (Table 5.8) and availability in large amounts as by-products of vegetable oils (1).

Sitosterols are the most abundant and widely distributed of plant sterols and occur in very complex mixtures comprising α-sitosterol (said to be in itself a mixture), β-sitosterol (stigmast-5-en-3β-o1) and γ-sitosterol; the last named is regarded as identical with clionasterol ($C_{29}H_{50}O$, m.p. 138°, $[\alpha]_D - 42°$) (2ab, 3) but according to Thomson *et al.* (4), it is a mixture of campesterol and β-sitosterol.

The major sources of β-sitosterol are cottonseed oil, sugarcane wax and tall oil. The most important source of γ-sitosterol is soysterols (see stigmasterol) which are being used for this purpose by Schering, A.G. and Searle of the U.S.A. (5,1).

Cottonseed oil may contain up to 1.6 per cent sterols; refined oils of American origin contain 0.6 to 1.0 per cent (6). Examination of cottonseed oil sterols showed that they consisted of 94-96 per cent of β-sitosterol (7,8).

Sugarcane was consists of large amount of β-sitosterol. Wax obtained from Ravalgaon Sugar Farm contained 10.2 per cent of sterol mixture, with β-sitosterol as the major constituent (9). In India sugarcane wax is extracted by two sugar factories, namely M/s Ravalgaon Sugar Farm Ltd., Ravalgaon (Maharashtra) and M/s K.C.P. Ltd., Vuvvuru (Andhra Pradesh) each with a capacity of about 275 kg/day during the season; but there is no regular production (10,11). The need to develop this potential source of β-sitosterol was emphasized in the 1977 Lucknow Symposium on Hormones and Oral Contraceptives. It is understood that some steroid drug manufacturers are showing interest in the use of β-sitosterol as starting material (12).

Tall oil, which is produced in the U.S.A. as a by-product of alkaline pulping of resinous wood such as pine, contains β-sitosterol in considerable amount in its unsaponifiable matter (13). Tall oil is not extracted in India although pines grow abundantly in Himalayan region.

Table 5.8 summarizes the microbiological transformations of cholesterol and phytosterols including sitosterols. For conversion of sitosterols to spironolactone see Fig.5.5. Sitosterols find use as precursor for estrogens and progestogens besides diuretics (Fig. 5.7).

REFERENCES TO SITOSTEROLS

1. Applezweig. Dioscorea - The pill crop, 1976.

2a. Dictionary of Organic Compounds, 1965, Vol. 5, 2902; 2b. Fifth and Cumulative Suppl., 1969, 857.

3. Brooks, C.J.W. Steroids: sterols and bile acids, *in* Rodd's Chemistry of Carbon Compounds, Vol. II, Part D, 1970, 154-55.

(*Contd on page* 137)

TABLE 5.8—MICROBIOLOGICAL CONVERSIONS OF CHOLESTEROL AND PHYTOSTEROLS*

Process and Patent	Conversions	Micro-organisms
Mitsubishi Chemical Industries Process—Arima *et al.*, U.S. Patent, **3, 388, 042** (1968), Priority Japanese application, 1964	Cholesterol, sitosterol and stigmasterol converted to AD (androstadiene) and ADD (androstadienedione) by action of a microorganism having a sterol decomposing ability in the presence of a compound capable of forming a chelate with iron and/or copper	Wide variety of micro-organisms: *Arthrobecter*, *Corynebacterium*, *Brevibacterium*, *Microbacterium*, *Protaminobacter*, *Bacillus*, *Serrotia*, *Nocardia*, *Mycobacterium* and *Streptomyces*. (*Chem. Abstr.*, 1966, **65**, 6262)
Gist-Brocades, Delft Process I—van der Waard *et al.*, British Patent, **1, 113, 887** (1970)	AD and ADD by fermentation in the presence of nickel, cadmium, cobalt, lead, and selenite ions	Micro-organisms of the genus *Mycobacterium*, *M. phlei* (Strain KNGSF 70) or *M. butyricum* (*Chem. Abstr.*, 1968, **68**, 114844; **69**, 27631; 1969, **71**, 57875)
Gist-Brocades, Delft Process II—van der Waard *et al.*, U.S. Patent, **3, 487, 907** (1970).	-do-	Micro-organisms other than Mycobacterium
Hungarian Process—Wix *et al.*, Hungarian Patent, **153, 831** (1965)	Cholesterol, ergosterol or stigmasterol to ADD by fermentation using 8-hydroxyquinoline as a chelating agent to inhibit cleavage of the steroid nucleus via 9-hydroxylation	*Mycobacteria* varieties *Mycobacterium phlei* (*Chem. Abstr.*, 1967, **67**, 107385)
Searele Process I—Kraychy *et al.*, U.S. Patent **3, 684, 657** (1972)	Cholesterol and phytosterols to AD and ADD	*Mycobacterium* sp. N.R.R.L.B - 3683 or enzymes therefrom (*Chem. Abstr.*, 1972, **77**, 124777).
Searle Process II—Marsheck & Kraychy, U.S. Patent, **3, 759, 791**, (1973)	Cholesterol and phytosterols converted to AD selectively	*Mycobacterium* sp. N.R.R.L.B - 3805 (*Chem. Abstr.*, 1973, **79**, 144867).

*Applezweig, Dioscorea—the pill crop, 1976

4. Thompson, M.J., Robbins, W.E. & Baker, G.L. The nonhomogeneity of soybean sterol, γ-sitosterol. *Steroids*, 1963, **2**, 505-12.
5. Kritchevsky, D. Sterols, *in* Comprehensive Biochemistry, Vol. 10, 1963, 13.
6. Bailey, A.E. Cottonseeds and Cottonseed Products, Their Chemistry and Chemical Technology (Interscience Publishers, Inc., New York), 1948, 380.
7. Wallis, E.S. & Chakravarti, R.N. The Nature of the steroids in cottonseed oil. *J. Org. Chem.*, 1937-38, **2**, 335-40.
8. Murti, K.S. & Achaya, K.T. Cottonseed Chemistry and Technology in its Setting in India (Publications & Information Directorate, CSIR, New Delhi), 1974, 299-300.
9. The Wealth of India — Raw Materials, Vol. IX, 1972, 161.
10. Mhaskar, V.V. & Kulkarni, A.B. Composition of sugarcane wax. *J. Sci. Ind. Res.*, 1956, **16B**, 374-75.
11. Draft Status Report on Utilization and Recycling of Wastes (Research, Development and Extension Requirements) (National Committee on Science and Technology, Government of India, New Delhi), 1975, 101.
12. Information from Dr Y.K. Hamied, Managing Director, CIPLA, Bombay.
13. Hillis, W.E. Wood Extractives and their Significance to the Pulp and paper Industries (Academic Press, New York), 1962, 400-01.

(see also Reference page 145).

CHOLESTEROL

Cholesterol (Fig.1.1) was one of the first materials to be used for the synthesis of steroid hormones by chemical reactions (1,2). With the discovery of cheaper and more versatile precursors, the use of cholesterol declined. The recent development of fermentation process which has virtually replaced intricate chemical degradation of cholesterol and the availability of cholesterol as a byproduct of various industries particularly wool grease industry, have revived interest in this precursor. A Japanese company, Mitsubishi Chemical Industries, has started producing steroid drugs by microbiological transformation of cholesterol (Table 5.8) (3,4). The French firm, Roussel, and the German firm, Schering, have been using this precursor as additional starting material for the conversion of dehydroepiandrosterone (DHA), an intermediate for the manufacture of estrone and 19-Nor compounds (1,2).

Commercial sources of cholesterol are sheep wool grease (15.0%), cattle spinal cord (4.0%) and cattle brain (2.1-3.6%) (1).

Extraction from Wool Grease—Sheep wool grease contains cholesterol in the form of its esters with fatty acids; besides it is associated with closely related lanosterol and triterpene alcohol (isocholesterol). These make the isolation and purification very difficult. The esters are hydrolysed by treating the wool grease with hot aqueous sodium or calcium hydroxide. The unsaponifiable fraction containing cholesterol is separated from soaps by extracting with acetone or ethylene chloride; care is taken to avoid emulsion formation. Cholesterol is then purified by fractional crystallization from alcohol or acetone, using activated charcoal as a decolourizing agent. The method is wasteful and although sheep wool grease contains 15 per cent of cholesterol, industrial yields are seldom more than 3 per cent.

Complex formation of cholesterol with hydrochloric, oxalic and perchloric acids is also used for purification. A complex of oxalic acid (1 mol. anhydrous) and cholesterol (2 mol.), formed by treating the ethylene chloride solution of sterol mixtures with anhydrous oxalic acid at 60°, crystallizes out in pure form. The complex is then decomposed in aqueous solution from which the cholesterol can be recovered by filtration or solvent extraction (1,2). For more details see monograph on cholesterol by Kritchevsky (5) and another one edited by Cook (6). For systematic chemical name and properties of cholesterol, see Table 5.1.

NBG Symposium on Raw Materials for Steroid Hormones and Oral Contraceptives held in March, 1977 recommended recovery of cholesterol from wool grease which is available in plenty in the country.

Extraction from Cattle Spinal Cord and Brain—Cholesterol, which is present in free form in these materials, is extracted by suitable solvents such as chlorinated hydrocarbons, esters, lower alcohols, and ketones (1).

Chopped raw spinal cord free from connective tissue is ground to a creamy pests. The paste is agitated with ethylene chloride at 29.5° for 2 hours and then allowed to settle. The lower solvent layer is tapped out and the material is re-extracted twice with equal volumes of the same solvent. The combined extracts are concentrated until the mixture boils at 87° and then cooled to 0°. Cholesterol crystallizes out; additional material is recovered from mother liquors. The combined material is purified by recrystallizing from ethylene chloride, using a decolorizer-filter aid, and then from alcohol. Colourless crystals have m.p. 148 to 149°. A tonne of raw spinal cord requires approximately 2500 litres of ethylene chloride (2500) lb raw spinal cord requires 750 U.S. gallon of ethylene chloride) for the first extraction; the same volume of solvent is rseequired for subsequent extractions.

Cholesterol of a high purity can be extracted from dried cattle brains by using acetone as a selective solvent (1).

In India, there are only two abattoirs—one in Deconar under the Greater Bombay Municipal Corporation and other at Tundla under the Ministry of Defence Production. Co-ordinated efforts have been initiated by the Biological Group of Laboratories of CSIR since 1975 to utilize the tissues from Tundla for production of cholesterol along with other products like insulin and pepsin (7).

For conversion of cholesterol, see Figs. 5.5 and 5.7. For microbiological transformation of cholesterol, see Table 5.8.

REFERENCES TO CHOLESTEROL

1. Applezweig. Steroid Drugs, 1962, 10, 32, 42-44.
2. Manning. *Trop. Prod. Inst. Rep.*, No. G 41, 1969, 10.
3. Blunden *et al.*, Trop. Sci., 1975, 17, 139-54.
4. Applezweig. Dioscorea — the pill crop, 1976.
5. Kritchevsky, D. Cholesterol (John Wiley and Sons, New york), 1958.
6. Cook, R.P. & Rattray, J.B.M. Methods of isolation and estimation of sterols, *in* Cholesterol, edited by Cook, R.P. (Academic Press, New York), 1958, 117-43.
7. Indian Pharmaceutical Guide, 1977 (Pamposh Publications, new Delhi), 14.

(see also Reference page 145).

DEOXYCHOLIC AND CHOLIC ACIDS

Deoxycholic and cholic acids (Table 5.1, Fig. 1.1) are the two important bile acids known to occur in the bile of different animals. Bile acids are derivatives of 5β- or 5α-cholan-24-oic acids or their analogues. Ox bile is the usual commercial source of deoxycholic and cholic acids. Hog bile, which contains 3α, 6α-dihydroxy and 3α, 6α, 7α-trihydroxy derivatives, has been used for this purpose in Canada (1).

Fresh ox bile contains 0.6 to 0.8 per cent of deoxycholic acid and 5 to 6 per cent of cholic acid (1,2). Both these acids occur as glycine and taurine conjugates in variable amounts; these conjugates are present as sodium salts, known as bile salts (3).

Inspissated bile, commonly known as bile paste, is the starting material for bile acids. Bile paste is obtained by evaporating fresh bile in open vessels to a solid concentration of usually 75 per cent. Trade in ox bile is almost entirely in the form of bile paste (25% moisture content). According to 1969 Market survey report a pound of bile paste cost about £ 7, i.e. $ 15 per kg (1).

Because of the demand for deoxycholic acid in some countries, it has become economical to convert cholic acid to deoxycholic acid. This can be done by either separating cholic acid from its deoxycholic acid from the bile acid misxture and then converting it to deoxycholic acid or directly converting cholic acid to deoxycholic acid from the bile acid mixture. Though both the methods are prevalent, the latter has an edge over the former since it avoids the separation of the two acids and is more economical (4).

Extraction of Deoxycholic Acid—The method described here is the preparation of deoxycholic acid from total bile acids. It involves selective oxidation of the 7-hydroxyl group to a ketone by treatment with an N-halosuccinimide, followed by a Wolff-Kishner reduction to give deoxycholic acid (4).

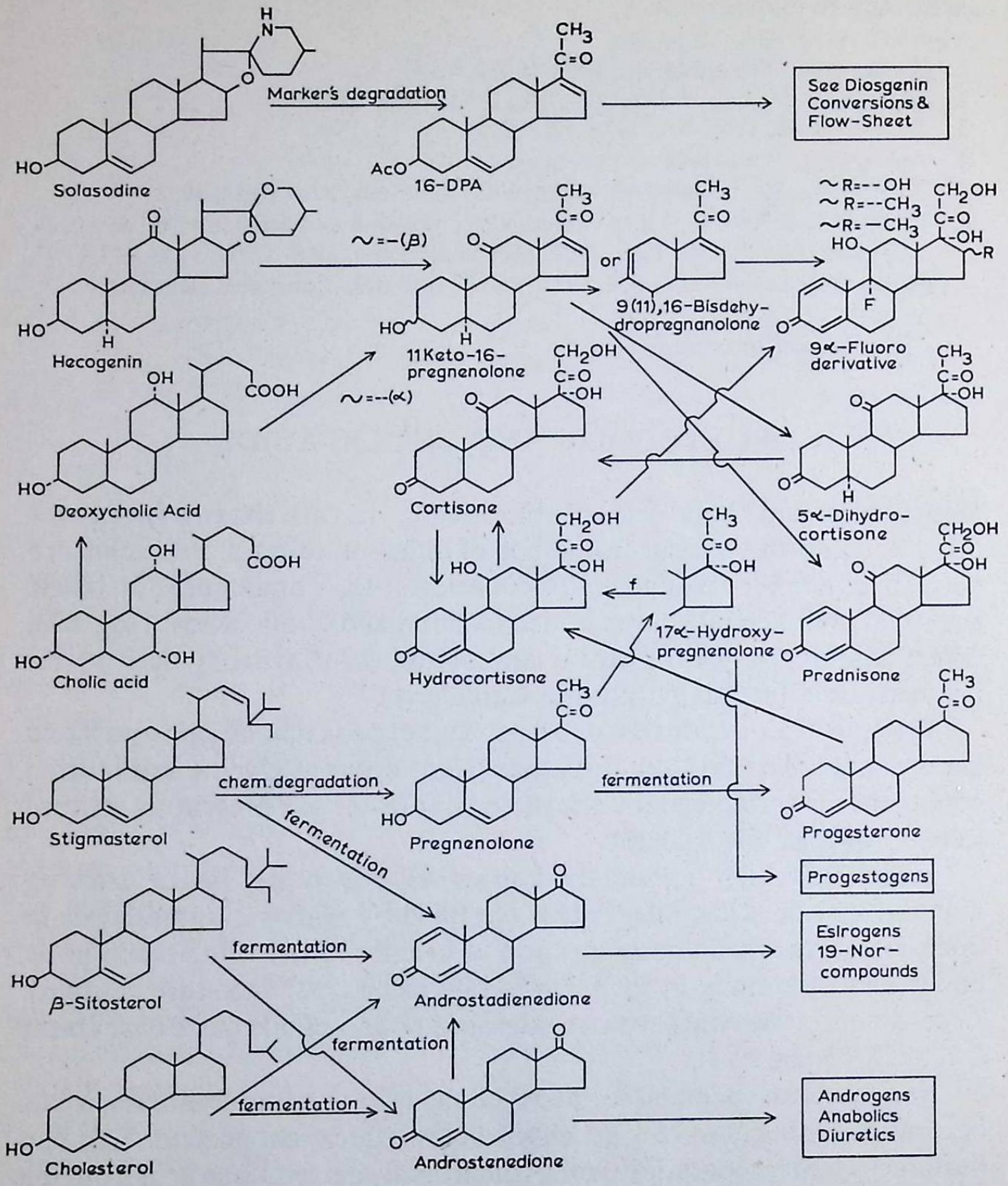

Solasodine
Marker's degradation
AcO
16-DPA
See Diosgenin Conversions & Flow-Sheet
Hecogenin
∼=-(β)
11 Keto-16-pregnenolone
or
9(11),16-Bisdehydropregnanolone
∼R=--OH
∼R=--CH3
∼R=-CH3
9α-Fluoro derivative
Deoxycholic Acid
COOH
∼=--(α)
Cortisone
5α-Dihydrocortisone
Cholic acid
Hydrocortisone
f
17α-Hydroxypregnenolone
Prednisone
Stigmasterol
chem. degradation
Pregnenolone
fermentation
Progesterone
Progestogens
Esirogens 19-Nor-compounds
β-Sitosterol
fermentation
Androstadienedione
fermentation
Cholesterol
fermentation
Androstenedione
Androgens Anabolics Diuretics

Fig. 5.7—Summary flow-sheet of industrial processes using steroid drug precursors other than diosgenin.

The bile paste suitably diluted is treated with sodium hydroxide to give 1.5 to 2.5N alkaline solution to release deoxycholic and cholic acids which are then precipitated by adding hydrochloric acid. The total bile acid precipitate (100g) is dissolved in water (1.5 litre) containing sodium bicarbonate (65g); and the solution treated with N-bromosuccinimide (56g) at room temperature for 24 hours. Acidification of this gives a precipitate which is dissolved in triethylene glycol (700 ml), to this 85 per cent hydrazine (110 ml) and potassium hydroxide (85g) are added. The mixture is distilled cautiously, until the temperature reaches 190°, after which it is refluxed for 3 hours. It is then cooled, diluted with water and acidified to precipitate deoxycholic acid which is in granular form and as such easily filterable. This crude material is purified by treatment with dry ether to form the deoxycholic acid etherate; pure acid from this complex can be obtained by heating in water and filtering. Yields of nearly 70 per cent can be obtained (4).

The process can also be applied to prepare deoxycholic acid from pure cholic acid. Bile acids are not used in India, as there are hardly any well-organized slaughter houses for oxen within the country; however, the above method was developed by an Indian and patented in the U.S.A.(4).

The objection to ox bile and bile acids as starting materials for steroid production is that ox bile is only a very minor by-product of the meat industry: Six steers of 500 kg average live weight will yield about 1 kg of fresh bile and 12 kg of fresh bile (i.e. from 72 steers) are required to obtain 1 kg of bile paste. The production of bile paste is dependent on the demand for meat and not on the demand of pharmaceutical industry (2).

Conversion—Shifting of the 12 oxygen to the 11 position, degradation of the bile chain to the necessary dihydroxyacetone grouping, and finally introduction of α,β-unsaturated ketone form the three major stages of conversion. However, as many as 25 to 30 chemical steps may be necessary to achieve synthesis of cortisone, hydrocortisone. Bile acid intermediate and finished products are summarized in Fig. 5.7.

As far as is known, bile acid process though lengthy is still operated by Merck and by Schering in U.S.A., Rouseel-Uclaf in France, Organon in the Netherlands, and one company in U.K. The French company is said to account for over one half of total world consumption of ox bile for the production of corticosteroids (5). The use of bile acids as starting materials for steroid drug manufacture is declining because they are in inadequate supply and the conversion process is out of date. If some companies are still persisting in their use of bile acids as raw materials, it may be due to the capital investments for the equipment which will have to be changed for other processes.

REFERENCES TO DEOXYCHOLIC AND CHOLIC ACIDS

1. Brooks, C.J.W. Steroids: sterols and bile acids, *in* Rodd's Chemistry of Carbon Compounds, Vol.II, Part D, 1970, 169, 171-73.
2. Manning. *Trop. Prod. Inst. Rep.*, No. G 41, 1969, 8.
3. Haslewood, G.A.D. Bile Salts, *in* Comprehensive Biochemistry, Vol. 10, 1963, 23-27.
4. Applezweig, Steroid Drugs, 1962, 44-46.
5. UNCTAD/GATT Markets, 1974, 79.

(see also Reference page 145).

ERGOSTEROL

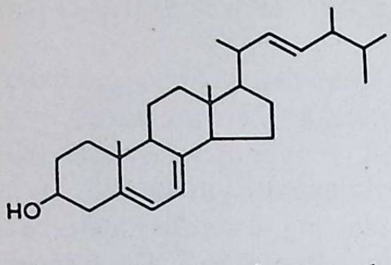


Fig. 5.8—Structure of ergosterol.

Ergosterol (Fig. 5.8, Table 5.1) is the most common of the fungal steroids called mycosterols. Although a readily available and cheap phytosterol, it has not found much favour with the steroid drug manufacturers (1). However, its microbiological transformation to ADD (Table 5.8) and its commercial availability as a by-product of the fermentation industry with extensive use in the production of Vitamin D (irradiated ergosterol) (3) cannot be overlooked.

Ergosterol occurs in most fungi including yeast, in lichens, algae and some vegetable oils (3). The main source of ergosterol is a yeast *Saccharomyces cerevisiae*. High yields up to 2.7% of the yeast (dry weight basis) can be obtained with certain strains grown in special aeriated medium (4).

Ergosterol may be recovered by treating the yeast cells with alkali, and extracting the resulting saponified mixture with a solvent such as ether. It can be purified by crystallization from ether (4). Another method is to heat a 15 per cent aqueous slurry of the yeast under pressure at 120° for 1/2 hour with sodium hydroxide in the presence of a partially water-soluble alcohol such as *n*-butanol. After filtration, ergosterol can be recovered by vacuum concentration of the butanol phase, addition of water, and crystallization (1).

Conversion—For conversion to progesterone, ergosterol is subjected to Oppenauer oxidation to obtain ergosterone which on treatment with hydrochloric acid in methanol yields methyl ether. Methyl ether on heating with hydrochloric acid gets converted to isoergosterone. Selective hydrogenation of 6,7 double bond in isoergosterone with hydrogen over palladium-carbon followed by ozonolysis which cleaves the 22, 23 double bond, yields the same aldehyde as in the case of stigmasterol (enamine route in Fig. 5.5). Rest of the steps remain same as for stigmasterol. The overall yield of progesterone from ergosterol is only 37 per cent (5).

REFERENCES TO ERGOSTEROL

1. Applezweig. Steroid Drugs, 1962, 42.
2. Applezweig. Dioscorea — the pill crop, 1976.
3. Heftman & Mosetting. Biochemistry of Steroids, 1960, 27-28, 94.
4. McGraw-Hill Encyclopedia of Science and Technology (McGraw-Hill Book Co. Inc., New York), Vol. 13, 1960, 138.
5. Fieser & Fieser. Steroids, 1959, 555-56.

(see also Reference page 145).

VEGETABLE PREGNANE STEROIDS

Pregnane steroids from a well-known gum oleoresin, named Indian Bdellium (*Guggul* of commerce) have been isolated in yields up to 3 per cent. The oleoresin occurs in a *Commiphora* sp. (1,2a). Although, like ergosterol, these steroids are not in the field, chances of their becoming precursors are not ruled out.

A process to obtain the useful steroids from oleoresin has been developed and patented by Sukh Dev *et al.*, under Indian Patent No.126354, 1975 (1). The method consists in extracting the oleoresin with a suitable organic solvent to furnish a product free from carbohydrate. The soluble material is processed to remove the essential oil and to separate the residue into various components. Alternatively the resin is saponified with alkali and the non-saponifiable portion separated into its constituents. In this way, cholesterol, a trihydroxy cholesterone and two isomeric pregnane steroids are isolated besides sesamin and other diterpenoids. These compounds have been obtained in good yields by fairly simple methods, thereby making this oleoresin a cheap source for these pregnane steroids.

The pregnane steroids can be suitably modified by simple steps into steroidal drugs of androgens/estrogens type (1).

It may be worth mentioning about the steroidal amines of the family *Apocynaceae*, particularly funtamine from *Funtumia latifolia* Stapf, and holaphyllamine from *Holarrhena africana* A.DC syn. *H. floribunda* Durand & Schinz. Both the amines are C_{21} steroids with α- and β-amino group at C-3 position respectively. Their remarkable resemblance to pregnane derivatives make them easily convertable to pregnenolone by replacing a primary amino group with a ketone. Because of their basic nature and attendant water solubility, extraction can be simpler than it is in the case of sapogenins. Furthermore, no hydrolysis stage is required as compounds do not occur as glycosides (3).

These amines have not received much attention apparently due to the difficulty in obtaining the plant material in exploitable amount. The above species are not reported in India; however another species of *Funtumia*, *F. elastica* Stapf, has been introduced into India in Nilgiris and Bangalore (2b).

REFERENCES TO PREGNANE STEROIDS

1. *CSIR News*, 1975, **25**. 24.
2a. The Wealth of India — Raw Materials, Vol. II, 1950, 313-14; 2b. Vol. IV, 1956, 93-94.
3. Applezweig. Steroid Drugs, 1962, 51-52.

(see also Reference page 145).

MISCELLANEOUS PRECURSORS

Smilagenin and its 25-*S* epimer sarsasapogenin, tigogenin and tomatidine have been explored for the production of steroid drugs (1).

Smilagenin can be obtained from *Smilax ornata* Hooker, *Yucca* spp. and the leaves of *Agave lecheguilla* Torr. Commercial exploitation was started in Mexico, and in 1968 smilagenin was reported to account for 1.5 per cent of raw materials used for steroid drugs, but more recent information would indicate a decline in its use (2). Smilagenin is unlikely to step in as steroid drug precursor in India; so is the case with sarsasapogenin.

Tigogenin is generally associated with hecogenin in sisal juice. It can be recovered and made use of in estrone manufacture (3).

Sarsasapogenin can be obtained from the seeds of *Yucca filifera* Hort. ex Engeln. In Mexico, it is planned to utilize the seeds as a source of sapogenin, edible oil and protein, since the plant is sufficiently abundant in an estimated area of 840,000 ha (4).

Tomatidine is the nitrogen analogue of neotigogenin and both have been found to occur together in primitive red currant tomato, *Lycopersicon pimpinellifolium* Mill. (5,6a). Tomatidine in the form of its glycoside tomatine is present in a number of *Lycopersicon* and *Solanum* species (6ab). It occurs in all varieties of *L. esculentum* Mill (Tomato); as the fruits ripen their tomatine content decreases, while the concentration in the leaves increases (5,6a).

Cordylagenin, cannigenin and brisbegenin, which can be obtained from the leaves of easily cultivated *Cordyline cannifolia* R. Br. and *C. stricta* Endl. are possible sources of steroids. Australia and Pacific islands are engaged in further development work in collaboration with Tropical Products Institute, London (4).

Conessine and holarrhimine, the *kurchi* alkaloids, present in the bark of *Holarrhena antidysenterica* Wall. have also been examined as possible starting materials for the production of 18-hydroxylated steroid hormones (5). The *kurchi* bark, which has long been used as a remedy for amoebic dysentery in India, has however, not found favour as steroid raw material for large scale production (7).

Any steroidal sapogenin or its N-analogue can become steroid drug precursor depending upon the availability of the plant raw material (3). However, it may already be too late for new raw material sources to be explored since according to Applezweig (1), the threat of steroid shortage appears to turn into almost a steroid glut.

REFERENCES TO MISCELLANEOUS PRECURSORS

1. Applezweig. Dioscorea — the pill crop, 1976.

2. UNCTAD/GATT Markets, 1974, 79.
3. Applezweig. Steroid Drugs, 1962, 50-52.
4. Blunden *et al.* *Trop. Sci.*, 1975, **17**, 139-54.
5. Heftman & Mosettig. Biochemistry of Steroids, 1960, 54, 56-57.

6a. The Wealth of India — Raw Materials, Vol. VI, 1962, 194-97; 6b. Vol. IX, 1972, 379; 6c. Vol. IV, 1956, 93-94.

7. Sarin, Y.K. Raw Materials for Indian Steroid Industry. *Indian Drugs*, 1974, **11**(10), 15-17.

REFERENCES

[References to individual precursors are given at appropriate places; here full particulars of the common references to Chapter 5 are given].

Applezweig, N. Dioscorea — the pill crop (Paper presented at the Annual Meeting of the Society for Economic Botany, University of Illinois, Urbana held on June 13-16, 1976).

Applezweig, N. Steroid Drugs (McGraw-Hill Book Co., Inc., New York), 1962.

Blunden G., Culling, C. & Jewers, K. Steroidal sapogenins: a review of actual and potential plant sources. *Trop. Sci.*, 1975, **17**, 139-54.

Comprehensive Biochemistry, Vol. 10 — Sterols, Bile Acids and Steroids, edited by Florkin, M. & Stotz, E.H. (Elsevier Publishing Co., New York), 1963.

Cultivation & Utilization of Medicinal and Aromatic Plants, edited by Atal, C.K. & Kapur, B.M. (Regional Research Laboratory, Jammu-Tawi), 1977, pp.590 (Medicinal plants: Steroidal, 9-38).

Dictionary of Organic Compounds, edited by Heilbron, I. and others (Eyre & Spottiswoode Publishers Ltd., London), 5 vols., 4th ed., 1965 — Fifth and Cumulative Supplement, edited by Stevens R., 1969.

Fieser, L.F. & Fieser, M. Steroids (Reinhold Publishing Corporation, New York), 1959.

Fieser, L.F. & Fieser, M. Topics in Organic Chemistry (Reinhold Publishing Corporation, New York), 1963.

Heftmann, E. & Mosettig, E. Biochemistry of Steroids (Reinhold Publishing Corporation, New York; Charles E. Tuttle Company, Tokyo), 1960, Modern Asia ed., 1961.

Proceedings of Second Workshop of AICIP (All India Co-ordinated Improvement Project) on Medicinal and Aromatic Plants, held at Institute of Agriculture, Gujarat Agricultural University, Anand on November 1-4, 1976, pp.233.

Manning, C.E.F. The market for steroid drug precursors with particular reference to diosgenin. *Trop. Prod. Inst. Rep.*, No. G 41, 1969.

NBG (National Botanic Gardens), Lucknow, Symposium on Production and Utilization of Vegetable Raw Materials for Steroid Hormones and Oral Contraceptives held on March 26-28, 1977. (Abstracts).

Rodd's Chemistry of Carbon Compounds, edited by Coffey, S. (Elsevier Publishing Company, Amsterdam, 2nd ed. Vol. II, Part D, 1970; Part E, 1971.

The Wealth of India — Raw Materials, A Dictionary of Indian Raw Materials, Vol. I-VI, (Council of Scientific & Industrial Research, New Delhi), 1948-1962; Vols. VII-XI (Publications & Information Directorate, CSIR, New Delhi), 1966-1976.

UNCTAD-GATT Markets — International Trade Centre. UNCTAD/GATT Markets for Selected Medicinal Plants and their Derivatives. Geneva, 1974. Chapter IV — Dioscorea and Diosgenin, 68-81, 146-49.

CHAPTER 6

PRODUCTION, REQUIREMENTS AND TRADE

Diosgenin

Mexico is the world's largest producer of diosgenin accounting for about three-fourth of the overall output (1). China, Guatemala, Costa Rica and India are the other producers. Puerto Rico, which was earlier producing diosgenin, has discontinued its production (2,3). Production of diosgenin in various countries is given in Table 6.1. The production figures after 1974 are not available.

The total world production of pharmaceuticals in the year 1974 was valued at about $ 21,000 million; of this, sex hormones and corticosteroid formulations totalled $ 1,320 million. This presupposes a world production of diosgenin and other precursors somewhere between 1000 and 1200 tonnes (2); assuming the production of 750 tonnes of diosgenin from Mexico and other countries, 450 tonnes equivalent might have come from other steroidal genins and sterols and also by total synthesis.

Mexico—The production of diosgenin in Mexico was 600 tonnes in 1974 but it declined thereafter on account of the regulatory measures adopted by the Mexican Government.

The manufacturers rely mainly on the supply of wild tubers, although some R&D work is being carried out on cultivation of *Dioscorea* spp. at Government insistance (1). To encourage domestic processing, the Government has prohibited the export of crude genin as well as the first intermediate, 16-DPA.

China—Production of diosgenin in 1968 was reported at 80 tonnes, but supplies do not appear to be available regularly in the international market (1).

TABLE 6.1—WORLD PRODUCTION OF DIOSGENIN[1]

(Quantity in tonnes)

Country	1963	1968	1974
Mexico	375	500	600[2]
Guatemala	10	30	—
Puerto Rico	—	20	nil
India	10	30	30
China	—	80	—
Total	395	660	Incomplete

[1] Applezweig, *Chem. Week*, May 17, 1969. [2] Applezweig, Dioscorea—The pill crop, 1976.

Guatemala—Production of diosgenin 1968 was 30 tonnes. The entire quantity was derived from wild sources. Several subsidiaries of foreign pharmaceutical companies are operating in the country(1).

Costa Rica—Production figures are not available.

India—The production of diosgenin in 1976 is estimated between 30 and 35 tonnes. Accurate data about annual production are not available.

Estimated production of diosgenin on the basis of the collections of wild dioscorea tubers during the preceeding years, with the assumption that diosgenin content was high in the initial years and comparatively low in the subsequent years, is given in Table 6.2. The total requirement of tubers for the installed capacity in the pharmaceutical industry is about 2,000 tonnes per year, but only 600 to 800 tonnes are available from natural sources (1).

Major producers of diosgenin in the country are Wyeth, CIPLA, CIBA-Geigy, Searle, all in Bombay and CIMPO Drug Factory in Jammu. There are also some small-scale extractors (Appendix III) (4,5).

Seven companies have been licensed in India for the production of synthetic hormones and intermediates; six of these, viz. Wyeth, CIBA-

TABLE 6.2—ESTIMATED DIOSGENIN PRODUCTION IN INDIA
(Quantity in tonnes)

Year	Dioscorea tubers wild	Diosgenin content, %	Diosgenin production*
1961-62	123	7	23.22
1962-63	804	5	8.61
1963-64	831	5	40.20
1964-65	708	5	41.55
1965-66	514	5	35.40
1966-67	677	5	25.70
1967-68	727	5	33.85
1968-69	586	4	36.35
1969-70	612	4	23.40
1970-71	570	4	24.48
1971-72	487	4	22.80
1972-73	712	4	19.48
1973-74	199	3	28.48
1974-75	-	-	5.97(24)‡
1975-76	521†	3	(26)‡
1976-77	-	-	15.63(30)‡

*The production data are based on the consumption figures of wild *Dioscorea* tubers.

†Since 1974-75, cultivated tubers are also being used, hence the actual production figures are higher.

‡Figures refer to rough estimated production according to Dr.Y.K. Hamied, CIPLA, Bombay.

Geigy, Searle, Glaxo Laboratories, Merck, Sharp & Dohme (M.S.D.) and CIPLA are in Bombay; and Organon in Calcutta (5,6a).

Wyeth, M.S.D., and Glaxo are engaged mainly in the production of corticosteroids; of these, the last two individually have a licensed capacity of 120 kg per year. Glaxo Laboratories base their production on 16-DPA purchased locally and to a very small extent on 16-DPA they manufacture from solasodine. M.S.D. on the other hand, import steroid intermediate for the manufacture of dexamethasone. Wyeth tops among the steroid manufacturers in both production and consumption of diosgenin. The company produces sex hormones also, particularly androgens including anabolics, and progestogens. CIPLA, CIBA-Geigy, Searle and Organon produce sex hormones and oral contraceptives, basing their production on diosgenin. The total annual licensed and installed capacities for the production of hormones in India were 6,468 kg and 6,720 kg respectively, in 1976 (5,6a).

Production of hormones (which includes corticosteroids) during 1973, according to the Hathi Committee Report, was about 2,262.65 kg in units having foreign equity of more than 50%, and 314.14 kg in wholly Indian units or units with equity participation up to 50% (2).

Actual break-down figures regarding the use of diosgenin individually for corticosteroids, sex hormones, oral contraceptives and intermediates are not available. However, on the basis of discussion with the major manufacturers of diosgenin and steroid drugs, as also on the basis of information available from other sources, the following pattern of consumption of diosgenin for 1976 appears to be logical: corticosteroids, 15; oral contraceptives, 6; and sex hormones including anabolics, and intermediates (only a small amount), 9 tonnes (4).

Other Steroid Precursors

Steroid drug precursors, other than diosgenin, being commercially produced include stigmasterol, bile acids, hecogenin, cholesterol and smilagenin. The country-wise consumption of these precursors during 1963 and 1968 is given in Table 6.3. The figures after 1968 are not available. On the basis of total world production of sex hormones and corticosteroids in 1974, it can be assumed that the consumption of these precursors along with total synthesis was around 450 tonnes during that year (3,2).

With the development of new sources and augmentation of the earlier ones, the production figures have possibly doubled since 1974. Japan has recently entered this field with enormous sources of cholesterol. In the U.S.A., sitosterol along with stigmasterol obtained from soyasterols is one of the cheapest steroid material available. In France and East Germany, appreciable quantities of steroid drugs are produced by total synthesis (3).

TABLE 6.3—WORLD CONSUMPTION OF STEROID PRECURSORS OTHER THAN DIOSGENIN*

Country	Nature of material	Quantity 1963	Consumed in tonnes 1968
Mexico	Smilagenin	—	10
United States	Stigmasterol	60	150
France	Bile acids	20	50
	Total synthesis	—	50
Germany	Cholesterol/Bile acids	5	10
Africa	Hecogenin	20	40
	Total	105	310

*Applezweig, *Chem. Week*, May 17, 1969.

REQUIREMENTS OF STEROID PRECURSORS

Applezweig considers diosgenin requirements in terms of two chief use-areas, viz., (i) corticoids and spironolactones, and (ii) contraceptives. On the basis of the production of 150 tonnes, the former is estimated to have consumed 1200 tonnes of diosgenin equivalent in 1976; this requirement is expected to increase by about 10 per cent annually in the next 10 years. In other words, the world requirements of corticoids and hence of diosgenin would double by 1985 (3).

According to Applezweig (3), production of oral contraceptives accounted for between 69 and 180 tonnes of diosgenin requirements for 1976; these figures are derived on the basis of women estimated to have used contraceptives with modern low dosage preparations amounting to 6.9 to 17.7 tonnes of oral contraceptives. As for the future demands, he estimates between 207 to 540 tonnes of diosgenin equivalent for 1985, i.e. 3 times expansion.

Estimated total world requirements for steroid drugs by 1985 are around 321 tonnes (92% corticosteroids, 8% oral contraceptives) and diosgenin equivalents 2607 tonnes.

Indian Requirements

Targets for requirements of steroid drugs in India are set at 15.34 tonnes which include 13.12 tonnes of corticosteroids for 1978-79, and after 5 years almost at double this quantity, 30.35 tonnes which include 25.77 tonnes of corticosteroids. Estimated diosgenin requirement is 122 tonnes for 1978-79 and 241.8 tonnes for 1983-84. These targets are probably set keeping in view the import substitution as also the exports of steroid drugs (2,7), since we are now in a position to provide from our cultivations sufficient additional source material to meet the needs of other countries, if a firm long range policy for *Dioscorea* and *Solanum* cultivation and selection is adopted (8).

TRADE

There is hardly any international trade in dioscorea tubers as they are extracted for diosgenin mostly in the countries of their origin. A few manufacturers in India, however, are believed to obtain some supply of wild tubers of *D. deltoidea* from Nepal (6a). In the same way most of the diosgenin is utilized in the country of its extraction. Only a small part of the diosgenin output of the world, chiefly from China, enters international trade (1); the Chinese diosgenin, however, is said to be of poor quality (6a).

Prices of Diosgenin

The prices of diosgenin, which have been rising every year reached the peak figures in 1975 and 1976 and then began a downward trend due to the competition with other precursors. China offered diosgenin at a price as low as $40 in 1977 December (6a). Table 6.4 gives the prices of dioscorea and diosgenin in India and Mexico during 1970-1978.

TABLE 6.4—PRICES OF DIOSCOREA AND DIOSGENIN IN INDIA AND MEXICO

Year*	Indian[1,2]		Mexican[3]	
	Dioscorea Rs/kg	Diosgenin Rs/kg	Dioscorea $/kg	Diosgenin $/kg
1970	4.50	122.06	0.360	11.25
1971	4.50	297.20	0.395	12.45
1972	4.50	450.00	0.415	14.52
1973	6.00	418.18	0.475	17.51
1974	6.00	848.34	0.810	27.68
1975	11.00	1100.76[4] (1306 for 95% pure)	1.622	54.78
1976	11.00	1000.00	5.600	152.20
1977	—	600.00[5]	0.95	95.00
1978	6.00	500.00[5] (750 for 95% pure)	—	40-50 (Chinese)[5]
Cultivated (1976)	3.00	800.00[6]	1.258	43.65

[1] Information from the Chief Conservator of Forests, J&K, Govt. Srinagar and also through personal communication with a purchaser.

[2] *Annu. Rep. CIMPO*, 1967-73, 276; 1974-75, 52; 1975-76, 26; 1976-77, 27.

[3] Applezweig, Dioscorea—the pill crop, 1976.

[4] Information from Shri B.C. Gulati, DRL, CIMPO, Jammu-Tawi.

[5] Information from Dr.Y.K. Hamied, CIPLA, Bombay.

[6] Information from Shri L.C. Rabha, RRL, Jorhat.

* Indian data pertains to financial year commencing from April, thus 1970 would mean April 1970 to March 1971.

The comparative costs of various steroidal raw materials and steroid drugs obtained from them are given in Table 6.5. The prices of some intermediates offered by well-known international firms, based on different raw materials, are summarized in Table 6.6.

The Government of India recently lifted the ban on imports of steroid drugs, as a result of which the prices of not only diosgenin but of the end-product hormones have crashed down. The liberalized import policy is, however, proving detrimental to the Indian steroid industry.

TABLE 6.5—COMPARATIVE COSTS OF STEROID RAW MATERIALS AND SOME PRODUCTS IN WORLD MARKET

Raw Materials	Cost $/kg	Contraceptive Steroids (Equiv Norethisterone) $/kg	Corticosteroids (Equiv. Hydrocortisone) $/kg
Diosgenin	15	150	90
	60	600	360
	120	1200	720
Hecogenin	120	—	720*
Stigmasterol	45	—	315
Stigmasterol	(Sitosterol recovered)		203
Sitosterol	4	84	—
Cholesterol	16	42	—
Ox Bile	10	—	50†

Note: Based on published prices of raw materials and yields of steroid drugs.

[1] Applezweig, Dioscorea—the pill crop, 1976.

*Useful mainly in production of dexamethasone and betamethanone.

†Bile process has highest labour and reagent cost.

TABLE 6.6—PRICES OF PRODUCTS FROM DIFFERENT RAW MATERIALS OF SOME WELLKNOWN STEROID MANUFACTURERS[1]

Manufacturer	Raw material	Intermediate product	Price (1976)
Upjohn (U.S.A.)	Stigmasterol	Progesterone	250 (350)*
Upjohn (U.S.A.)	Stigmasterol	17-Hydroxyprogesterone	310 (875)*
Searle (U.S.A.)	Sitosterol	Androstenedione	135 (500)*
Schering (West Germany)	Sitosterol	Androstadienedione	325 (700)*
Roussel (France)	Total synthesis	Norandrostenedione	1,000 (1,400)*
Jenapharm (East Germany)	Total synthesis	Diverse	(*c*.50% higher)*

[1] Applezweig, Dioscorea—the pill crop, 1976.

*These figures are for the intermediate product obtained from Mexican diostenin.

Imports and Exports

India was not importing diosgenin or other steroid drug precursors because of the import restrictions, though a small amount of bile intermediate and some steroid drugs are imported (6a,9). During 1973-74 India imported steroid drugs worth Rs 0.500 million, besides India imports some balancing chemicals like lithium metal, tetrahydrofuran, dimethyl sulphoxide, etc., used in the manufacture of steroid drugs (2,6a).

To encourage domestic processing, the Government of India had imposed a ban on the exports of dioscorea tubers as also on diosgenin (2). Small quantities of intermediate namely, 16-DPA, pregnenolone, DHA-acetate, androstenedione and expoxypregnenolone and very small amount (4 to 5 kg) of finished product, methandienone (an anabolic), are exported to Japan, U.K., Federal Republic of Germany, Brazil, Italy and Switzerland. Exports of these steroids during the period 1974-76 amounted to about Rs 0.500 million (4).

TRENDS

Steroid Starting Materials

The chief groups of products that are synthesized from diosgenin are corticosteroids and spironolactones (diuretics), which account for 80 per cent of all steroids (10); according to Applezweig, 95 per cent (3). Hecogenin is being obtained in greater quantities from sisal wastes from Africa for corticosteroids by Glaxo particularly for sophisticated drugs like Betamethasone. Haiti may provide large amounts of hecogenin. For dexamethasone, Merck can use the hecogenin intermediate. Stigmasterol is another raw material alternative being commercially used in U.S.A. for corticosteroid. Bile acids, though of limited availability are still in use for this purpose. Solasodine is also being used. It is thus seen that diosgenin, which enjoyed supremacy in this field for long, is now facing competition from other starting materials (3).

The second major area of steroid production is oral contraceptives. Until recently Mexican diosgenin has been the sole source material for contraceptive steroids with the exception of *dl*-norgestrel which is totally synthesized. In 1975, Mexican Government nationalized diosgenin production and raised the price to above that of total syntheses with the result manufacturers of sex hormones switched over to the alternative raw materials with better routes of production, offering cheaper products (3).

Mitsubishi Chemical Industries (MCI) of Japan with their progressive microbiological products from conventional starting material like cholesterol, which is available in large quantities from the wool grease of the Japan wool processing industry, have almost turned the tables on diosgenin. MCI also used squalene as raw material for microbiological transformation to steroid drugs (3).

Sitosterol is comparatively a new entry in the field of steroid starting materials but with large amounts available from the separation of stigmasterol from soysterols in the U.S.A. and in West Germany and easier microbiological degradation to androstenes leading not only to androgens but also to estrogens, progrestogens and spiranoloctone, Mexican diosgenin has almost lost grounds in contraceptive field and in diuretics (3). Further, total synthesis from petroleum has become increasingly important in France, and East and West Germany. Also active in this field are Hungary, Switzerland, China, the U.S.A. and the U.K. Dr.C. Djerassi (10), the celebrated "Father of 19-nor contraceptives" and an authority on steroid reactions, feels changeover of sources for oral contraceptive steroids is complete. According to him, likely future pattern for steroid contraceptives is that, apart from norgestrel, half will be made by total synthesis and the remainder will continue to be semi-synthetic with microbiological processes becoming increasingly important.

Mr. Applezweig (3), another authority on steroid drugs, has similar views. In his opinion, even if the Mexican Government reverses its policy and returns to prices prevalent some years ago, it no longer seems possible that diosgenin can be considered a material of choice for 19-nor contraceptives, estrogens or diuretics.

Trends in India

While the international scene has shifted from diosgenin to other steroid drug precursors including new ones like sitosterol and squalene, diosgenin appears to be going strong in India, with the advent of successful cultivation of *D. composita* and *D. floribunda*, although high-yielding varieties and mutants of *Solanum khasianum* have also been developed and hecogenin as by-product from sisal industry is making its appearance felt.

Wyeth, which takes a lion's share of diosgenin in India, will continue to use this genin in coming years; CIBA has plans to extend the cultivation of *D. composita* up to 120 hectares or so by 1978-79, and will thus depend on diosgenin only. CIPLA will also be extending its cultivation to 40 hectares, and may use diosgenin though solasodine, cholesterol, sitosterol and total synthesis will also form basis for their steroid drug manufacture.

Glaxo, which is the first in the country to use solasodine will continue to use this material more and more for their betamethasone, as the farmers are showing interest in the easy-to-cultivate cash crop of *Solanum khasianum.* Glaxo will also use hecogenin.

Merck, Sharp & Doehm which use imported intermediate as the starting material for dexamethasone can use 16-DPA from diosgenin or hecogenin intermediates from hecogenin, but definite plans of this firm are not known. This is also the case with Organon.

Searle, which has started cultivating *D. composita* at Bangalore and may probably do so in Goa, appears to be relying mostly on diosgenin from their farm, but may purchase from others as well. In this context, one is tempted to mention that Searle (U.S.A.) has developed a competitive industry to Mexican diosgenin with respect to androgens and diuretics from sitosterol as the starting material. It is, however, too early to predict whether Searle (India) will use this material since its commercial recovery is yet to be achieved in India.

Large scale isolation of sitosterol from sugarcane press mud, stigmasterol from soybean (still in its infancy in India) and cholesterol from wool grease has been strongly recommended at the NBG Symposium held at Lucknow in March, 1977. Although some R&D work is in progress on cholesterol, it is difficult to predict at this stage how long it will take these precursors to become industrial sources of steroid drugs in India. Normally it takes 5 to 10 years for translating pure research into an industrial application.

Pregnane steroids easily obtainable from *Commiphora* species appear to posses good prospects as steroid raw materials, since they can be suitably modified by simple steps into androgens and estrogens. A process has already been patented for the isolation of these compounds.

Synthetic Steroids

The steroid drug precursors are facing increasing competition from total synthesis. According to some authorities, wide-spread commercial synthesis of steroids with petroleum as a starting material will eventually displace all other sources, especially in the production of contraceptives (11). However, since total synthesis of corticosteroids is not economically feasible with existing methods, natural materials remain the major source of world steroid production (3).

According to a 1968 estimate, about 13 per cent of world's supply of steroid drug contraceptives are derived from total synthesis. Synthetic production is known to have been introduced in West Germany, East Germany, France, Hungary, Switzerland, China, the United States and the United Kingdom.

In India, total synthesis of steroids has not yet been undertaken commercially, although a great deal of R&D work has been done. The Indian Council of Medical Research is supporting a collaborative study on the synthetic steroids for "pills" at various centres. Messrs. Unichem Laboratories and CIPLA are actively interested in the total synthesis of steroids, particularly estrone and *dl*-norgestrel (6a,b). *dl*-Norgestrel (13-ethyl-19-norethisterone) is the most potent progestogen available today and is widely prescribed of its kind on a world-wide basis. It was synthesized by the Wyeth Laboratories of the U.S. and was first introduced in 1968 (12,6b). India imports this steroid for incorporation in contraceptive pills (13,4).

It appears that contraceptive pills of the future will be almost entirely obtained by total synthesis, although cholesterol may also be used to some extent. Mention may be made of squalene, a precursor of cholesterol and a petroleum fraction, which is being converted to AD and ADD by microbiological transformation by Mitsubishi of Japan (3).

Nonsteroidal Products

As steroid drugs become more complicated, the cost of synthesizing them partially or totally increases out of proportion to starting material cost. The emphasis is therefore shifting to the hunt for new and better drugs (14), which may lead to usage of nonsteroidal products. Of the nonsteroidal products, the most important ones that may pose a threat to diosgenin and other steroid drug precursors are prostaglandins (PG) which are a family of closely related C_{20} fatty acid derivatives possessing a five-membered ring. They are extremely potent substances with simpler molecular structure occurring in tissues of a great variety of living organisms. Some of the important prostaglandins are PGE_1, PGE_2, PGA_1 and PGF_2 (15,16).

Areas of clinical utility of these compounds include: asthma (PGE_1, PGE_2), anti-inflammatory applications, gastric anti-secretory (PGE_1, PGA_1), lowering of blood pressure without the usual decrease in renal function (PGA_1); labour induction and abortion (PGE_2 and PGF_2); and contraception (PGF_2). These uses overlap with those of various steroid drugs which, however, will be always necessary for substitution therapy (16,17).

According to UNCTAD/GATT Market 1974 (1), only one prostaglandin preparation appears to be marketed, in Ireland, for use in preparation for delivery. As per the latest information, prostaglandins are being used for medical termination of pregancies. A prostaglandin preparation called Ono 802 has been synthesized by a Japanese pharmaceutical firm which can be used for the evacuation of fertilized ovum and is undergoing fullscale clinical trials. Prostaglandins and their mechanism of action are subject of much investigations and hold a bright and exciting future not only in academic research but also for practical utility. Pharmaceutical industry appears to have high expectations from the market preparations based on natural or synthetic prostaglandins, which may become a large scale reality before long.

Besides prostaglandins, nonsteroidal estrogens have been discovered and are in use since long. They include *trans* form of diethylstilbestrol (stilbestrol) (*cis* form is less active), the *meso* form of the saturated analogue hexestrol, dienestrol and benzestrol. Of all the nonsteroidal estrogens the most potent is diethyl stilbestrol. It is as active as estradiol and in contrast to the natural estrogens, it is highly active when given by mouth and the duration of action is longer. Several compounds of stilbestrol series are orally effective. Owing to its cheapness, diethylstilbestrol is used exclusively in the meat and poultry industry (18).

The nonsteroidal products pose a threat to steroid drugs and thus to the precursors including diosgenin. However, in the immediate future there is no such danger, although manufacturers will have to watch further developments in prostaglandin field and study the demand aspect.

REFERENCES

1. International Trade Centre UNCTAD/GATT Markets for Selected Medicinal Plants and their Derivatives. Geneva, 1974. Ch. IV—Dioscorea and Diosgenin, 70-81.
2. Export Potential of Selected Medicinal Plants & their Derivatives (Basic Chemicals, Pharmaceuticals and Cosmetic Export Promotion Council, Bombay), 1976, 39-41.
3. Applezweig, N. Dioscorea—the pill crop (Paper presented at the Annual Meeting of the Society for Economic Botany, Univ. of Illinois at Urbana on June 13-16, 1976).
4. Personal communication or discussions with steroid drug manufacturers.
5. Indian Pharmaceutical Guide 1977 (Pamposh Publication, New Delhi), 12-13.

6a. Information from Dr. Y.K. Hamied, Managing Director, CIPLA, Bombay; 6b. Hamied, Y.K. Progress and prospects of the steroid industry in India (Lecture delivered at the Indian Institute of Science, Bangalore on March 8, 1976).

7. Information from Shri I. Sundaresh, Dy. Secretary, Basic Chemicals, Pharmaceuticals & Export Promotion Council, Bombay.
8. Kunjithapadam, S. Utilization of various raw materials for the production of steroids, *in* Cultivation & Utilisation of Medicinal and Aromatic Plants, edited by Atal, C.K.& Kapur, B.M. (Regional Research Laboratory, Jammu), 1977, 9-20.
9. Information from the Director General, Department of Commercial Intelligence & Statistics, Calcutta.
10. Djerassi, C. Contraceptives of the future (Lecture delivered at the Royal Society, London, Meeting held on February 18-19, 1976).
11. Blunden, G., Culling, C. & Jewers, K. Steroidal sapogenins: a review of actual and potential plant sources. *Trop. Sci.*, 1975, 17, 139-54.
12. Chemistry in the Economy (American Chem. Society, Washington, D.C.), 1973, 182.
13. Guidelines for Industries, 1976-77. (Government of India, Department of Industrial Development, Ministry of Industry & Civil Supplies, New Delhi), 315-20.
14. Applezweig, N. Steroid Drugs, (McGraw-Hill Book Co. Inc., New York), 1962, 35.
15. von Euler, U.S. & Eliasson, R. Medicinal Chemistry, Vol. 8—Prostaglandins (Academic Press, Inc., New York), 1967, 7-39.
16. Butt, W.R. Hormone Chemistry, Vol. 2. Steroids, Thyroid Hormones, Biogenic Amines and Prostaglandins (Ellis Horwood Ltd., Chichester). 2nd (Revised) ed., 1977, 243-56.
17. Weeks, J.R. The Prostaglandins: their nature, formation and general pharmacology, *in* Prostaglandins and Cyclic AMP—Biological actions and clinical applications, edited by Kahn, R.H. & Lands, W.E.M. (Academic Press, Inc., New York), 1973, 7-9, 14.
18. Astwood, E.B. Estrogens and progestins, *in* the Pharmacological Basis of Therapeutics, edited by Goodman, L.S. & Gilman, A. (The Macmillan Co., New York) 3rd ed., 1965, 1541.

APPENDIX I

Plant Species Reported to Contain Diosgenin

Plant Species	Reference
Agave angustiarum Trelease	1a
A cantala Roxb.	2a
A. ellemeetiana H. Jacobson	3
Aletris farinosa Linn.	4a
Balanites aegyptiaca Delile syn. *B. roxburghii* Planch	4d, 5a, 6
B. orbicularis Sprague	7
B. pedicellaris Mildbr. & Schlecht.	5b
B. wilsoniana Dave & Sprague	5b
Chamaelerium carolianianum Willd.	4b
Clintonia borealis Rafin.	4b
Costus speciosus Sim.	8
Daucus carota Linn.	25
Dioscorea alata Linn.	10
D. althaeoides R.Knuth	11
D. asclepiadea Prain & Burk	10,12
D. balcanica Kosanin	10,12
D. barlettii Morton	10
D. belizensis Lundell (see also *D. hondurensis* R. Knuth)	11
D. bulbifera Linn.	4c, 10
D. capillaris Hemsl.	4c, 10
D. caucasica Lipsky	10
D. collettii Hook. f.	10,13
D. composita Hemsl. (see also *D. macrostachya* Benth.)	1a-e, 10
D. cyphocarpa Robins.	10
D. daemona Roxb. (see also *D. hispida* Dennst.)	14
D. deltoidea Wall.	15a
—var. *orbiculata* Prain & Burk.	11
D. dugesii Robins.	10
D. esculenta Burk.	10,15ab
D. floribunda Mart. & Gal.	10
D. floridana Bartlett .	4d
D. friedrichsthalii R. Knuth	10
D. glauca Muhl.	1b, 16
D. gracillima Miq.	10, 12
D. grandifolia Schlecht.	10
D. hirsuticaulis Robins.	4b,14
D. hirticaulis Bartlett	4b
D. hispida Dennst. syn. *D. daemona* Roxb.	10
D. hondurensis R. Knuth syn. *D. belizensis* Lundell	17
D. izuensis Akahori	10, 12
D. jaliscana S. Wats.	10
D. japonica Thunb.	17
D. lobata Uline	10

D. macrostachya Benth.	10
D. mexicana Guill.	10
D. militaris Robins.	10
D. minima Robins. & Seaton	10
D. multinervis Benth.	10
D. nelsonii Uline	10
D. nigrescens R. Knuth	11
D. nipponica Makino	10
D. panthaica Prain & Burk.	10
D. pentaphylla Linn.	14
D. platycolpota Uline	10
D. plumifera Robins.	10
D. polygonoides Humb. & Bonpl.	10
D. polystachya Turcz.	10
D. prazeri Prain & Burk.	15ab
D. pringlei Robins.	10
D. quaternate J.F. Gmel.	10
D. quinqueloba Thunb.	10
D. remotiflora Kunth	10
D. septemloba Thunb.	10
D. sititoana Honda et Jotani	10
D. spiculiflora Hemsl.	10
D. subtomentosa Miranda	10
D. sylvatica Ecklon	10
D. tenuipes Franch. & Sav.	10
D. tepinapensis Uline	10
D. testudinaria R. Knuth	10
D. tokoro Makino	10
D. ulinei Greenm.	10
D. urceolata Uline	10
D. villosa Linn.	10
D. zingiberensis C.H. Wright	11
Kallstroemia pubescens (G. Don) Dandy	2b
Momordica charantia Linn.	9b
Nicotiana tabacum Linn.	18
Nolina erumpens S. Wats.	4c
N. greeni (?)	4c
Paris polyphylla Smith	19
Pseudophoenix insignis O.F. Cook	1d
Smilacena stellata Desf.	4c
Smilax china Linn.	20a
S. perfoliata Lour.	20a
Solanum aviculare Forst. f. syn. *S. Laciniatum* Ait.	2
S. dulcamara Linn.	20a
S. elaeagnifolium Cav.	20a
S. indicum Linn.	20a
S. khasianum C.B. Clarke emend. Sen Gupta	20a

S. laciniatum Ait. (see also *S. aviculare*)	21
S. nigrum Linn.	21
S. pinnatum Cav.	21
S. surattense Burm. f. syn. *S. xanthocarpum* Schrad. & Wendl.	20a
S. vespertilio Ait.	21
Tofieldia gramnifolia (?)	4b
Tribulus terrestris Linn.	20b
Trigonella corniculata Linn.	20b
T. foenum-graecum Linn.	20b, 22
Trillium declinatum Rafin.	4d
T. erectum Linn.	4d
T. govanianum Wall.	23
T. grandiflorum Salisb.	4b
T. hugeri Small	4d
T. ludovicianum Harbinson	4d
T. recurvatum Beck	4d
T. sessile Linn. syn. *californicum* Kellogg	4c
T. simile Gleason	4d
T. stylosum Nutt.	4c
T. vaseyi Harbinson	4d
Yucca filamentosa Linn.	1c, 20c
Y. gloriosa Linn.	20c

1a Wall *et al.*, *J. Am. Pharm. Assoc., Sci. Ed.*, 1957, **46**, 653; 1b. ibid., 1954, **43**, 503; 1c. ibid., 1954, **43**, 1; 1d. ibid., 1955, **44**, 438; 1e. *J. Pharm. Sci.*, 1961, **50**, 1001.

2a Chakravarti *et al.*, *Bull. Calcutta Sch. Trop. Med.*, 1959, **7**, 5; 2a. *J. Inst. Chem. India*, 1976, **48**, 170.

3 Blunden *et al.*, *Trop. Sci.*, 1975, **17**, 139.

4a Marker *et al*, *J. Am. Chem. Soc.*, 1940, **62**, 2620; 4b. ibid., 1942, **64**, 1283; 4c. ibid., 1943, **65**, 1199; 4d. ibid., 1947, **69**, 2242.

5a Hardman & Sofowara, *Econ. Bot.*, 1972, **26**, 169; 5b. Hardman, *Trop. Sci.*, 1969, **11**, 196.

6 Dhekne & Bhide, *J. Indian Chem. Soc.*, 1951, **28**, 588.

7 Hardman &Wood, *Phytochemistry*, 1971, **10**, 887.

8 Dasgupta & Pande, *Experientia*, 1970, **26**, 475.

9a Khanna *et al.*, *Indian J. Exp. Biol.*, 1977, **15**, 586; 9b. ibid., 1973, **11**, 58.

10 Coursey, D.G., Yams (Longmans, Green & Co. Ltd, London), 1967, 216.

11 Gerasimenko & Tropova, *Rastit. Resur.*, 1971, **7**, 192.

12 Akahori, *Phytochemistry*, 1965, **4**, 97.

13 Cheng, *Econ. Bot.*, 1965, **19**, 1.

14 Karnick, *Planta Med.*, 1968, **16**, 269.

15a Barua *et al.*, *J. Indian Chem. Soc.*, 1956, **33**, 799; 15b. ibid., 1954, **31**, 173.

16 Chakravarti & Dash, *Bull. Calcutta Sch. Trop. Med.*, 1960, **8**, 59.

17 Gerasimenko, *Rastit. Resur.*, 1972, **8**, 161.

18 Turner, *Indian Drugs*, 1977, **14**(6), 112.

19 Singh *et al.*, *Indian J. Chem.*, 1966, **4**, 460.

20a The Wealth of India—Raw Materials (Publications & Information Directorate, CSIR, New Delhi), Vol. IX, 1972, 366, 378, 380, 382, 383, 394; 20b. Vol. X, 1976, 284, 299, 303, 304; 20c. Vol. XI, 1976, 15, 16.

21 Puri & Bhatnagar, *Pharmacos*, 1974, **19**, 7.

22 Fazli & Hardman, *Trop. Sci.*, 1968, **10**, 66.

23 Sarin & Kapoor, *Indian J. Pharm.*, 1965, **27**, 6.

APPENDIX II

Glossary of Botanical and Agricultural Terms

Acuminate	tapering to an acute end.
Allotetraploid	an allopolyploid formed by the doubling of the chromosome number in a diploid hybrid, i.e. a tetraploid with a diploid set of chromosomes from each parent.
Basal	attached to or arising from the base.
Bifid	divided into two parts half way down.
Bract	a modified reduced leaf from the axil of which a flower or floral axis arises.
Bulbil	a small, usually axillary bulb.
Capsule	a dry dehiscent fruit, opening by two or more valves.
Chartaceous	paper-like in texture.
Clone	a group of individuals resulting from vegetative multiplication; any plant propagated vegetatively and therefore presumably a duplicate of its parent.
Coriaceous	leathery, thick and tough.
Cordate	heart-shaped, i.e. more or less deeply notched at the base and in form like a conventional heart.
Culm	the stem, usually hollow, of bamboos and grasses.
Cuspidate	tipped with a cusp (a sharp, usually rigid terminal point or break).
Cyme	an inflorescence in which the main axis and all the lateral axes are each terminated by a flower and the flowering proceeds outwards and downwards.
Deltoid	shaped like an equilateral triangle.
Dentate	sharply toothed, especially with teeth not pointing forward or backward.
Digitate	spreading like the fingers of a hand; in a compound leaf, when the leaflets are borne at the apex of the petiole.
Dioecious	where the sexes occur on different individuals, the male flower on distinct plant from the female, e.g. in Papaya.
Diploid	having the somatic number of chromosomes (2DC), characteristic of the species.
Divaricate	spreading widely apart.
Drupe	a fruit with a more or less succulent flesh enclosing a single, 1-many-celled stone, such as a plum.
Embryogenetic	relating to the process leading the formation of the embryo.
Fascicle	a close cluster or bundle.
Genome	the chromosome set, especially when considered genetically.
Germplasm	in early theories of inheritance, a spefcific nuclear substance which was supposed to be the bearer of the hereditary characters and remain unchanged in spite of the differentiation of the body.

Glabrous	smooth, without any kind of hairs.
Globose	somewhat spherical.
Glomerate	collected into heads.
Harrow	a type of light cultivator without wheels; it is dragged over the ground for the purpose of pulverising and levelling it, or for covering seeds.
Hirsute	bearing long and somewhat stiff hairs.
Hispid	with rough or bristly hairs.
Hoe	tool for loosening soil, scraping up weeds, etc.
Homeologus	a hybrid with a number of genomes as in wheat.
Imbricate	overlapping
Lamina	the blade of a leaf.
Lanceolate	shaped like a lance-head; narrowly ovate and tapering to both ends.
Lobe	any division of an organ, or specially a rounded division or projection.
Meiosis	reduction division of chromosomes.
Mutagens	the chemicals or γ-rays causing heritable variation.
Mutant	plant which has undergone mutation.
Mutation	inception of a heritable variation.
Ob	used as a prefix, inverts the term affixed. Thus an ovate leaf has the wider part towards the base, an obovate leaf is inversely ovate and has the wider part towards the apex.
Obcordate	heart-shaped, but with the notch at the apex.
Oblong	longer than broad and with the sides more or less parallel.
Obovate	inverted ovate.
Orbicular	circular in outline
Ovate	egg-shaped, with a broader base.
Panicle	a loose compound inflorescence with usually the main axis racemose, the secondary and tertiary axes racemose or not.
Perennial	a plant which lives for several years, not dying after flowering and fruiting once.
Perianth	the floral envelopes, calyx and corolla both; a term mainly used when there is little or no difference in size and appearance between the sepals and petals.
Petiolate	furnished with petiole.
Petiole	the stalk of a leaf.
Polyploid	having more than twice the normal haploid ($\frac{1}{2}$ the diploid) number of chromosomes.
Puberulous	slightly hairy with very short hairs.
Raceme	an (indefinite or centripetal) inflorescence in which the main axis continues to grow, the loswest flowers being the oldest, opening first.
Rhizome	an underground stem, usually horizontal and elongated.
Sessile	without a stalk.

Spike	an inflorescence with sessile flowers on an usually elongate axis.
Stamen	the floral organ bearing the anther and pollens.
Stigma	the part of the pistil which receives the pollen.
Stipitate	having a stipe or stock.
Tetraploid	possessing twice the normal number of chromosomes.
Variegation	diversification in colours.

APPENDIX III

Diosgenin Extraction Centres in India

Diosgenin is extracted by the following pharmaceutical firms and laboratories in their factories in India:

1. Wyeth Laboratories Limited
   NA. 146, Shastri Marg, Ghatkopar
   Bombay 400086

2. Chemical, Industrial & Pharmaceutical
   Laboratories Limited, Vikhroli
   Bombay 400079

3. CIPLA Research Centre & Factory
   Virgonagar P & T Office
   Bangalore 560049

4. CIBA-Geigy of India Ltd
   Bhandup
   Bombay 400078

5. Drug Research Laboratory (CIMPO)
   (Farms and Factories J & K)
   Jammu-Tawi 180001

6. Scopa Pvt. Ltd, Jammu-Tawi

7. Bela Products Pvt. Ltd
   7/A, Modern Industrial Estate
   Bahadur Garh 124507
   Haryana

8. M/s Ganga Ram Suraj Prakash
   Manufacturers of Fine Chemicals,
   Extracts, Intermediates & Oleoresins
   Opp. Octroi Post, Basti Sheikh
   Jullundur City 144001

9. Nayar Minerals Export Ltd
   Damtal
   Himachal Pradesh

10. Goanka Lab., Jaipur 302001

11. Mentha Pharmacy, Amritsar 143001

A commercial plant is being set up in West Bengal by the Directorate of Cinchona and Other Medicinal Plants of the West Bengal Government at Mongpoo (Darjeeling Dist.). One small scale unit in Uttar Pradesh is being set up or is already set up.

INDEX

(Botanical names of plants are given in italics)

17α-Acetoxy progesterone, 99, 102, 109
AD (Androstenedione), 94, 96, 102 130, 133-36, 140, 151
ADD (Androstadienedione), 94, 96, 103, 112, 133-36, 140, 142
Adrenal cortex steroids, 1, 3, 5, 97
Agave (spp.), 125, 127, 157, see also Sisal
A. ameniensis, 127
A. americana, 127
A. angustifolia, 126, 127, 157
A. cantala, 126, 127, 157
A. decipiens, 127
A. ellemeetiana, 47, 157
A. elongata, 127
A. fourcroydes, see *A. rigida*
A. lecheguilla, 144
A. mirasdorensis, 127
A. nirvana, 127
A. rigida, 125
A. sisalana, 125, 127, 129
A. vera-cruz, 126, 127
A. versehaftelu, 127
A. wightii, see *A. angustifolia*
Aletris farinosa, 157
Aldosterone, 97
Algae, 142
Anabolics, 1, 8, 106, 108, 140, 148
Androgens, 106, 108, 133, 140, 153, 154
Androstadienedione, see ADD
Androstenes, 95, 99, 153
Androstenedione, see AD
Antifertility agent, 1, 106
Apocynaceae, 143
Assay procedures, 60, 75-90

B

Balanites (spp.), 11, 47, 61, 66, 157
B. aegyptiaca, 23, 47, 53-56, 65, 67, 83, 88, 157
B. orbicularis, 47, 56, 157
B. pedicellaris, 47, 157
B. roxburghiana, see *B. aegyptiaca*
B. wilsoniana, 47, 157
Bdellium, Indian, 143
Benzestrol, 155
Betamethasone, 94, 97, 98, 107, 128, 151-53
Bile acids, 1, 3, 6, 7, 112, 139, 140, 148, 149
Bile, inspissated, 139
Bile, ox, 151
Bile paste, 139, 140
Bombay aloe, 126
Brisbegenin, 144

C

Calabar bean, 132
Callus culture, 46
Cannigenin, 144
Cantala, 125
17α-Caproyloxy progesterone, 99, 102, 109
Cattle brain, 138
Cattle spinal cord, 138
Chamaelerium carolianianum, 157
Chlormadinone acetate, 102, 109
Cholesterol, 1, 4, 7, 9, 52, 112, 114, 130, 136-38, 140, 149, 151-55
Cholic acid, see also Bile acids, 2, 3, 112, 114, 139-41
Clintonia borealis, 157
Colorimetric methods, 77, 78, 89
Column chromatography, 74, 75, 77, 86-89
Commiphora, 143, 154
Compound S, see Substance S
Conessine, 144
Contraceptives, 153, 154
Contraceptive pill, 1, 6, 8, 148, 151, 152, 154
Contraceptive steroid, 151, 152, 154
Cordylagenin, 144

Cordyline cannifolia, 144
C. stricta, 144
Corticoids, see Corticosteroids
Corticosteroids, 1, 94, 95, 97-101, 106, 108, 128, 134, 146, 147, 149, 151, 152, 154
Cortisol, see Hydrocortisone
Cortisone, 1, 5, 6, 94, 95, 97, 98, 100, 106, 107, 128, 140, 141
Costus Linn., 11, 89
C. speciosus, 9, 23, 47-52, 60, 65, 66, 89, 157
Cotton seed oil, 135
Cowpea, 133

D

Daucus carota, 157
Dehydroepiandrosterone, see DHA
  acetate, see DHA acetate
6-Dehydromedroxyprogesterone, 110
16-Dehydropregnenolone acetate, see 16-DPA
  oxime, see 16-DPA oxime
Densitometric TLC, 75, 81, 89
Deoxycholic acid, 2, 3, 112, 114, 139-41, see also Bile acids
Deoxycorticosterone, 94, 97, 106, 108
  acetate, 98
Desert date, 53
Dexamethasone, 94, 97, 98, 106, 107, 148, 152, 153
DHA (Dehydroepiandrosterone), 137
  acetate, 94, 96, 99, 102, 103, 152
Dienestrol, 155
Diethylstilbestrol, 155, 156
Dioscin, 60, 62
Dioscorea(s), see *Dioscorea*
Dioscorea (spp.), 3, 7, 8, 11-13, 21-47, 50, 51, 66, 89, 100, 112, 157, 158
D. alata, 11, 41
D. althaeoides, 12, 157
D. balcanica, 12, 157
D. belizensis, 12, 157
trade, 150
D. clarkei, see *D. prazeri*
D. composita (=*D. macrostachya*), 6, 9, 12, 21, 25, 26, 31-33, 37-43, 60, 65, 68, 71, 75-77, 80, 81, 83, 153, 154, 157
D. deltoidea, 8, 9, 12-18, 21, 24, 34-47, 60, 69-71, 73, 75, 78-80, 83, 150, 157
  var. *orbiculata*, 12, 157
  var. *sikkimensis, see D. prazeri*
D. floribunda, 7, 9, 12, 21-31, 33, 37-46, 60, 61, 65, 69-71, 75-77, 80, 81, 83, 88, 122, 123, 153, 157
D. friedrichsthalii, 12, 21, 25, 39, 40-42, 157
D. glauca, 12, 157
D. gracillima, 60, 157
D. hondurensis, 12, 157
D. macrostachya, 31, 32, 158, see also *D. composita*
D. mexicana, 5, 6, 12, 13, 158
D. nigrescens, 12, 158
D. panthaica, 12, 13, 158
D. prazeri, 8, 13, 18-23, 32, 36, 37, 41, 60, 75-77, 158
D. sikkimensis, see *D. prazeri*
D. spiculiflora, 12, 13, 24, 25, 39, 40, 41, 158
D. sylvatica, 12, 39, 40, 41, 80, 158
D. tokoro, 5, 158
D. zingiberensis, 12, 13, 158
Diosgenin, 1-3, 5-9, 11-13, 15-18, 20-24, 26, 29-37, 41-56, 60-90, 93-96, 98, 100, 112, 121-24, 128, 131, 134, 146-56
Diosgenin
  assay procedures, 60, 75-90
  conversions, 93-103
  extraction, 61-67
  properties, 72
  purification, 73, 74
    83, 84, 87, 88
  requirements:
    India, 149, 150
    world, 149
  screening methods, 73
  sources, 11

Diosgenin (*Contd*)
  trends: India, 153
    world, 152
ψ-Diosgenin diacetate, 93, 100
Diosone, 93, 100
Diuretics, see Spironolactones
Dolichos lablab, 133
16-DPA (16-Dehydropregnenolone acetate), 93-95, 98, 99, 100, 101, 140, 146, 148, 152, 153
  oxime, 94, 96

E

EPA (Epoxypregnenolone acetate), 94, 96, 100, 152
16,17-EPA; 16,17-Epoxide; Epoxy acetate; Epoxypregnenolone acetate, see EPA
Ergosterol, 2, 3, 112, 114, 136, 142, 143
Estradiol, 1, 3, 6, 94, 99, 109, 156
Estrenes, 95, 96, 99, 103
Estrogens, 106, 109, 133, 135, 136, 140, 153-54
  nonsteroidal, 155
Estrone, 1, 3, 4, 94, 99, 103, 109, 144, 154
Ethinylestradiol, 94, 99, 103, 109-10
Ethinylestrenol, 94, 103, 110
Ethisterone, 6, 130
Ethynylestradiol, see Ethinylestradiol
Ethynylestradiol, see Ethinylestradiol
Ethynyl testosterone, 99

F

Fenugreek, 54, 61, 62, 66, 67, 74-76, 84,89, see also *Trigonella foenum-graecum*
Fermentation (Microbiological conversion), 7, 96, 97, 100-102, 104, 105, 134-36, 152
Fludrocortisone, see Fluorocortisol
9α-Fluorocorticosteroids, 98, 101
  16-substituted, 101, 130
9α-Fluorocortisol (Fludrocortisone), 94, 106, 108
  acetate, 98, 101, 107
9α-Fluoroprednisolone, 101, 133
Funtamine, 143
Funtumia elastica, 143
F. latifolia, 143
Furostadiene derivative, 93

G

Gas liquid chromatography, see GLC
Genetic improvement, 117
Genetics, 40
Genin, steroidal, 3, 145, 148
Gitogenin, 84, 87, 89, 90
GLC, 73, 75, 79-81
Glucocorticoids, 97
Glycine max (*G. soja*), 132
Gracillin, 60
Gravimetric methods, 76, 89

H

Hechtia texensis, 125
Hecogenin, 1-3, 7, 9, 112, 114, 125-31, 144, 148, 149, 151-53
Heconin, 128, 129
Hemisaponin, 128
Hexestrol, 155
Holaphyllamine, 143
Holarrhena africana, 143
H. antidysenterica, 144
H. floribunda, see *H. africana*
Hormones
  sex, see Sex hormones
  steroid, 4
  synthetic, 147
Hybridization, 40, 117
Hydrocortisone (Cortisol), 1, 6, 94, 97, 98, 100, 106-107, 133, 140
  acetate, 100, 130, 141
17α-Hydroxypregnenolone, 133, 140
11α-Hydroxyprogesterone, 133

11-Hydroxyprogesterone, 6, 105
17α-Hydroxyprogesterone, 94, 99, 102

I

Indian bdellium, 143
Intermediates, steroid, 8, 94-96, 133, 148
IR-Spectrophotometry, 75, 82-89

K

Kallstroemia Scop., 11, 89
K. pubescens, 9, 23, 47, 51, 60, 65, 66, 158
11-Ketopregnenolone, 130, 140
11-Ketotigogenin, 130
Kurchi alkaloids, 144

L

Lablab bean, 133
Leptadenia reticulata, 133
Lichens, 142
Liliaceae, 112
Lycopersicon esculentum, 144
L. pimpinellifolium, 144

M

Medroxyprogesterone, 109, 110
Meiotic studies, 43
Mestranol, 109, 110
Methanedienone, 94, 99, 102, 108, 152
16α-Methyl-prednisolone, 7α-bromo, 98
    7α-chloro, 98
17α-Methyltestosterone, 96, 99, 102, 108
Microbiological conversion, see Fermentation
Mineralocorticoids, 97
Momordica charantia, 158
Mutation breeding, 43, 117, 118
Mycosterols, 142

N

Neotigogenin, 74, 87-90, 114
Nicotiana tabacum, 158
Nolina spp., 158
Nonsteroidal estrogens, 155
Nonsteroidal products, 155
19-Nor contraceptives, 153
    steroids, 95, 99, 103, 133
    super-hormones, 6
Norethindrone, see 19-Norethisterone
19-Norethisterone, 99, 103, 110
Norethynodrel, 94, 103, 106, 110
dl-Norgestrel, 110, 152, 154
Norgestrel, 153
19-Nors, see 19-Nor steroids
19-Nortestosterone, 94, 99, 103, 108

O

Oil, cotton seed, 135
    soybean, 132
    tall, 135
Oral contraceptive, see contraceptive pill
Ordeal bean, 132
Ox bile, 139, 151

P

Paris polyphylla, 47, 158
Pennogenin, 60, 61
Perhydrocyclopentanophenanthrene, 1, 2
PG, see Prostoglandins
Physostigma venenosum, 132
Phytosterols, 136
Precursors, steroid drug, 1, 2, 7, 112, 114, 152-56
    nonsteroidal, 112
Prednisolone, 6, 94, 97, 98, 100, 106, 107, 133
Prednisone, 6, 94, 97, 98, 100, 106, 107, 128
Pregnane steroids, 143, 154
Pregnenes, 95, 98, 102

Pregnenolone, 94, 96, 99, 109, 143, 152
  acetate, 130, 133
Preparative thin layer chromatography, see *P*-TLC
Press mud, sugarcane, 154
Progesterone, 1, 3, 4, 94, 98, 99, 102, 105, 130, 132-34, 142
Progestogens, 106, 109, 135, 140, 153, 154
Prostaglandins (PG), 155, 156
Pseudodiosgenin, see ψ-diosgenin
Pseudophoenix insignis, 158

S

Saccharomyces cerevisiae, 142
Sapogenin, 3, 125, 144,
Sapogenins, steroidal, 3, 144
Sarsasapogenin, 90, 143
Sex hormones, 1, 3, 4, 6, 8, 106, 146, 148, 152
Sheep wool grease, 137
Single-node leaf-cutting, 29, 44
Sisal, 125-29, 152, 153,
  see also *Agave* spp
Sitosterol(s), 1, 2, 3, 7, 56, 132, 135, 136, 151, 153, 154
β-Sitosterol, 2, 73, 114, 130, 135, 140
γ-Sitosterol, 130, 135
Smilagenin, 90, 144, 148, 151
Smilacena stellata, 158
Smilax ornata, 144
S. china, 158
S. perfoliata, 158
Solamargine, 113
Solanaceae,112
Solanum alkaloids, 113
Solanum (spp.), 115, 123, 124, 150, 158, 159
S. aviculare, 113, 115, 123, 158
S. elaeagnifolium, 115, 123, 158
S. incanum, 115
S. indicum, 115, 158
S. khasianum, 113, 115-24, 153, 158
  var. *chatterjeeanum*, 113-24, 159
S. laciniatum, 115, 123, 159
S. mammosum, 115
S. myriacanthum, 113
S. nigrum, 115, 159
S. platanifolium, 113, 115
S. reflexum, 113
S. robustum, 115
S. surattense, 115, 159
S. trilobatum, 115
S. verbascifolium, 115
S. viarum, 113
Ṣolasodine, 1, 2, 7, 9, 112-24, 140, 148, 152, 153
Soybean, 132, 133, 153
Soysterols, 135, 153
Spironolactones (Diuretics), 8, 99, 108, 130, 133, 135, 140, 149, 152-54
25*R*-Spirosta-3:5-diene, 62
Squalene, 65, 112, 152, 155
Stereoparents (Steroid parents), 95, 96
Steroid
  drugs, 1, 106-10, 146, 148, 149, 151-56
  hormones, 4
  intermediates, 94-96, 133, 148
  nucleus, 1, 2
  parents, 95, 96
  pregnane, 143
  processes, 100-103, 130
  synthetic, 154
Sterols, 1, 130, 146
Stigmasterol, 1-3, 7, 56, 73, 112, 114, 130, 132-36, 140, 151-54
Substance S (Compound S), 94, 96, 97, 100, 101, 104, 105
Sugarcane press mud, 154
Sugarcane wax, 135
Synthetic steroids, 154

T

Tall oil, 135
Testosterone, 1, 3, 4, 94, 96, 99, 102, 108
Thin layer chromatography, see TLC

Tigogenin, 74, 87-90, 114, 125, 144, 160, 163
Tissue culture, 44-47
TLC, 73-75, 87, 89
    densitometric, 75, 81, 89
P-TLC, 72, 73, 75, 89
Tofieldia gramnifolia, 159
Tomatidine, 144
Tomatine, 144
Triamcinolone, 94, 97, 98, 106, 107, 133
    acetonide, 97, 107, 133
Tribulus Linn., 11
T. terrestris, 159
Trigonella (spp.), 11, 159
T. foenum-graecum, 23, 47, 51, 52, 60, 83, 86, 88, 159, see also Fenugreek
Trillarin, 60
Trillin, 60
Trillium (spp.), 159
T. erectum, 60, 159
Triolone triacetate, 94, 96, 98, 100

V

Vigna unguiculata, 133

W

Wax, sugarcane, 135
Wool grease, 137, 152, 154

Y

Yam, 3, 5, 8, 11, 36, 79, 80
Yamogenin, 52, 53, 55, 56, 61, 65, 72-74, 82-90
Yeast, 142
Yucca (spp.), 125, 144, 159
Y. filifera, 144

000143